EPIDEMIOLOGY OF LARGE COLON VOLVULUS

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Master of Philosophy

By

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ABSTRACT

Epidemiology of Large Colon Volvulus

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Colic is a major cause of mortality in the horse. It is most commonly related to gastrointestinal pathology and has significant economic and welfare implications. Large colon volvulus (LCV) is a painful and rapidly fatal form of colic and in some hospital populations represents between 10 and 20% of horses with colic that undergo exploratory laparotomy. There is a paucity of information on survival following LCV and on risk factors associated with the occurrence of LCV.

The aims of this thesis were firstly to describe the long-term survival of horses with LCV and to identify factors associated with survival, and secondly to determine risk factors for development of LCV. The first study presented in Chapter Two utilised clinical data and long-term follow up information from horses with LCV undergoing general anaesthesia at a UK equine hospital over a ten-year period. The study population comprised 116 horses. Of these, 77% survived general anaesthesia. Of the horses that survived anaesthesia, the percentage that survived to discharge, to one year, and to two years was 70.7%, 48.3% and 33.7% respectively. Median survival time of horses that survived general anaesthesia was 365 days. Survival time was negatively associated with higher pre-operative packed cell volume, colon serosal colour, increasing heart rate at 48 hours post-operatively, and evidence of colic during post-operative hospitalisation.

Chapter Three presents the results of a prospective case-control study, which aimed to identify horse- and management-level risk factors associated with LCV. Cases were recruited from 4 clinics in the UK and controls were randomly selected from the client population of these clinics over a 24-month period. Multivariable logistic regression was used to investigate variables associated with increased risk of LCV. Increasing height, multiple colic episodes in the previous 12 months and mares, with a greater odds ratio in mares that had previously foaled, were found to increase the risk of LCV. Receiving medication, (excluding anthelmintic treatment) in the previous 7 days, and quidding behavior were also associated with increased risk. Management-level variables associated with greater risk of LCV were an increase in the hours of stabling in the previous 14 days, an increasing number of horses on the premises, and 3 or more people involved in the horse’s care. Nutritional variables associated with increased risk were being fed hay, being fed sugar-beet, a change in pasture in the previous 28 days, and an alteration in the amount of forage fed in the last 7 days.

In conclusion, the studies within this thesis have improved our ability to prognosticate survival in horses with LCV and allowed us to identify individuals at increased risk of this disease. Importantly, the work has also identified a number of management practices, which might be modified, in an attempt to reduce the incidence of LCV. This thesis has highlighted several areas that merit on-going research, and which may, in the future, further contribute to our understanding of the disease. This research is relevant to clinicians and horse owners, particularly owners whose horses undergo surgery to correct a LCV, or owners whose horses’ are at increased risk of LCV, such as owners of horses that have suffered previous colic episodes or stud-farm managers.

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CHAPTER 1

Introduction and Literature Review

Introduction

Colic is a term used to describe abdominal pain, which in the horse, is most often related to the gastrointestinal system. It is an important disease of the horse, both in terms of equine welfare and economics, and in some horse populations is the most common cause of mortality [1, 2]. Large colon volvulus (LCV) is a painful and often fatal cause of colic in the horse [3] and is reported to affect 10-20% of colic cases that undergo surgery [4, 5]. Volvulus greater than or equal to 360 degrees has been found to be significantly associated with poor survival and post-operative complications [6, 7], with survival to hospital discharge following LCV reported to lie between 35-74% [8, 9, 10, 11]. Survival following LCV is related to the degree of vascular compromise of the large colon and the severity of the subsequent systemic inflammatory response [6].

There have been numerous epidemiological studies investigating horse and management level risk factors associated with colic in the horse [12, 13, 14, 15, 16, 17, 18, 19, 20, 21]; however, there are no publications investigating specific risk factors for LCV. Identification of modifiable risk factors for LCV might allow the implementation of disease prevention strategies to reduce the incidence of the disease.

Figure 1: *Intra-operative photographic image of distended oedematous large colon in a horse with a large colon volvulus.*

Gross anatomy of the large colon

The equine large intestine is comprised of the caecum, the large (or ascending) colon (Fig.1), the transverse colon, the small colon and the rectum. The large colon is composed of two U-shaped lengths of intestine and is 3 to 3.7m in length, with a capacity of over 100 litres [22]. It originates on the right side of the abdomen, at the caecocolic junction, and is subdivided into the right ventral colon, the left ventral colon, the left dorsal colon, and the right dorsal colon [23] (Fig. 2).

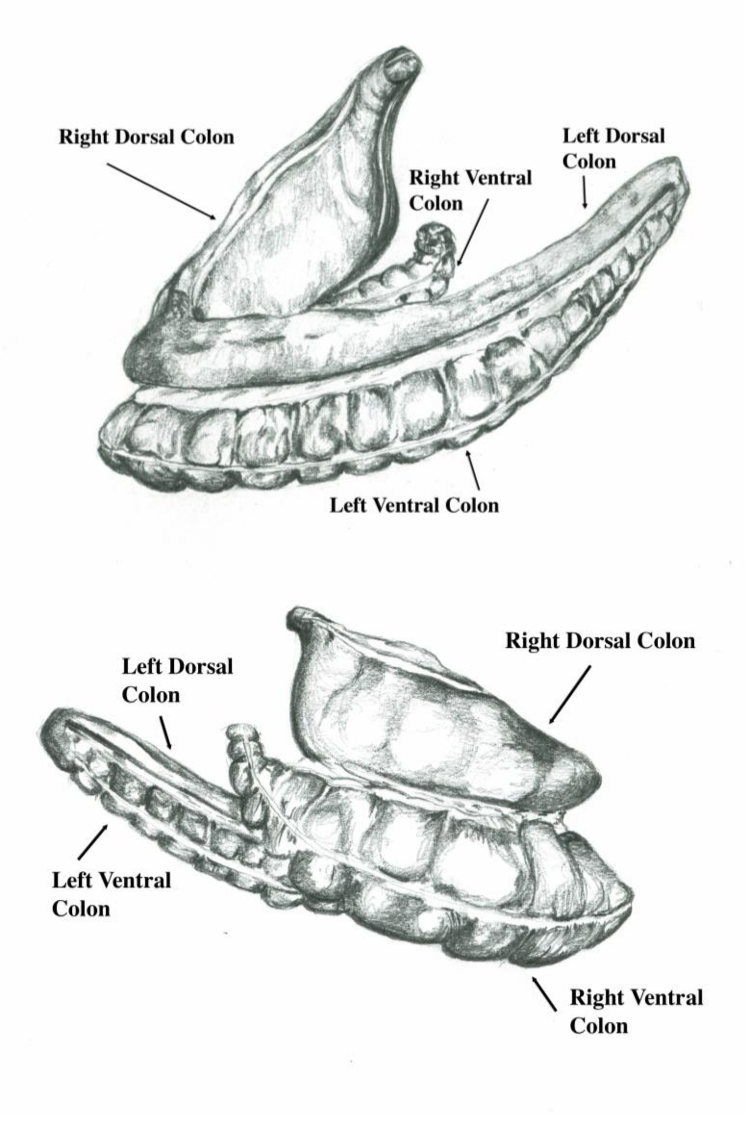


Figure 2: *Two drawings of the large colon in its normal orientation, as viewed from the left side of the horse (above) and the right side of the horse (below). The horse's head is to the left in the picture above and to the right in the picture below.*

The right ventral colon is attached to the caecum via the caecocolic fold, which is continuous with the lateral band of caecum [24]. It extends cranio-ventrally from the caecum and turns caudally at the sternal flexure to become the left ventral colon. The left ventral colon continues to the caudal extent of the abdomen to reach the pelvic flexure (Fig. 2 and 3). The left and right ventral colons have a diameter of approximately 25cm, and narrow to approximately 8cm at the pelvic flexure [22]. The left dorsal colon extends cranially from the pelvic flexure to the level of the diaphragm, where it becomes the right dorsal colon at the diaphragmatic flexure. The large colon terminates in the right side of the abdomen at the junction of the right dorsal colon with the transverse colon [22]. The diameter of the large intestine reduces significantly at this point from approximately 50cm in the right dorsal colon to approximately 8cm in the transverse colon [22]. The ventral and dorsal areas of colon are connected by short mesentery (Fig. 3), the ascending mesocolon, which originates from the right side of the root of mesentery [24]. The only anchoring attachments of the large colon are the caecocolic fold and the mesocolic attachment to the root of the mesentery [25].

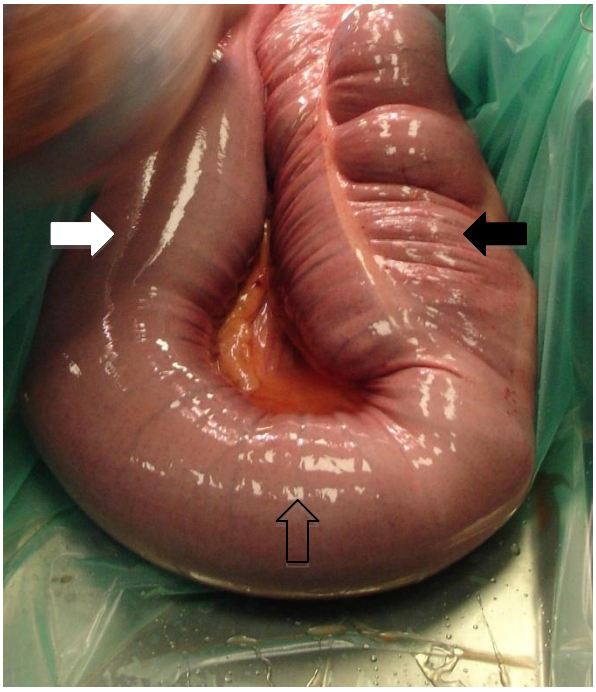


Figure 3. *Intra-operative photographic image of a horse's large colon. The distal aspect of the left ventral colon (black arrow), the pelvic flexure (arrow with no fill), and the proximal left dorsal colon (white arrow) can be seen. The ventral colon is saculated with two of the four taenial bands visible. The dorsal colon is smooth with no taenial bands visible. The ascending mesocolon can be seen to connect the ventral and dorsal colons.*

Both the right and left ventral colons are sacculated and have four taenial bands; two dorsal bands within the mesentery and two free ventral bands [23]. The colonic vasculature is associated with the dorso-medial band [24]. The dorsal colons are not sacculated. The left dorsal colon has one taenial band and the right dorsal colon has three taenial bands, one of which lies within the mesocolon.

Microscopic anatomy of the large colon

The wall of the large colon, similar to the rest of the digestive tract, is composed of the tunica mucosa, tunica submucosa, tunica muscularis and the tunica serosa. The tunica mucosa consists of the superficial epithelium, the lamina propria and the muscularis mucosae. Unlike the small intestine, the mucosal surface of the large intestine is devoid of villi [26]. Simple columnar epithelial cells line the luminal surface [22]. The surface is punctuated by straight tubular glands or crypts, which extend into the muscularis mucosae, and contain a large number of goblet cells [22, 26]. The lamina propria contains numerous lymphoid aggregates. The tunica muscularis consists of an inner circular layer and outer longitudinal layer of smooth muscle. The outer longitudinal layer of smooth muscle is comparatively thin, except where it forms the taenial bands [22]. These are discrete aggregations of smooth muscle and connective tissue. They provide mechanical support to the colon, are involved in the maintenance of the orientation of the colon within the abdomen, and allow distension and contraction of the colon [24]. They are composed of varying proportions of smooth muscle and connective tissue, the proportions of which relate to their function [27].

Vascular supply of the large colon

The blood supply to the large colon is derived from cranial mesenteric artery, which branches to form the ileocaecocolic artery and the right colic artery (Fig. 4). The colic branch of the ileocaecocolic artery supplies the ventral colon to pelvic flexure, where it joins the right colic artery (Fig. 4) [25].

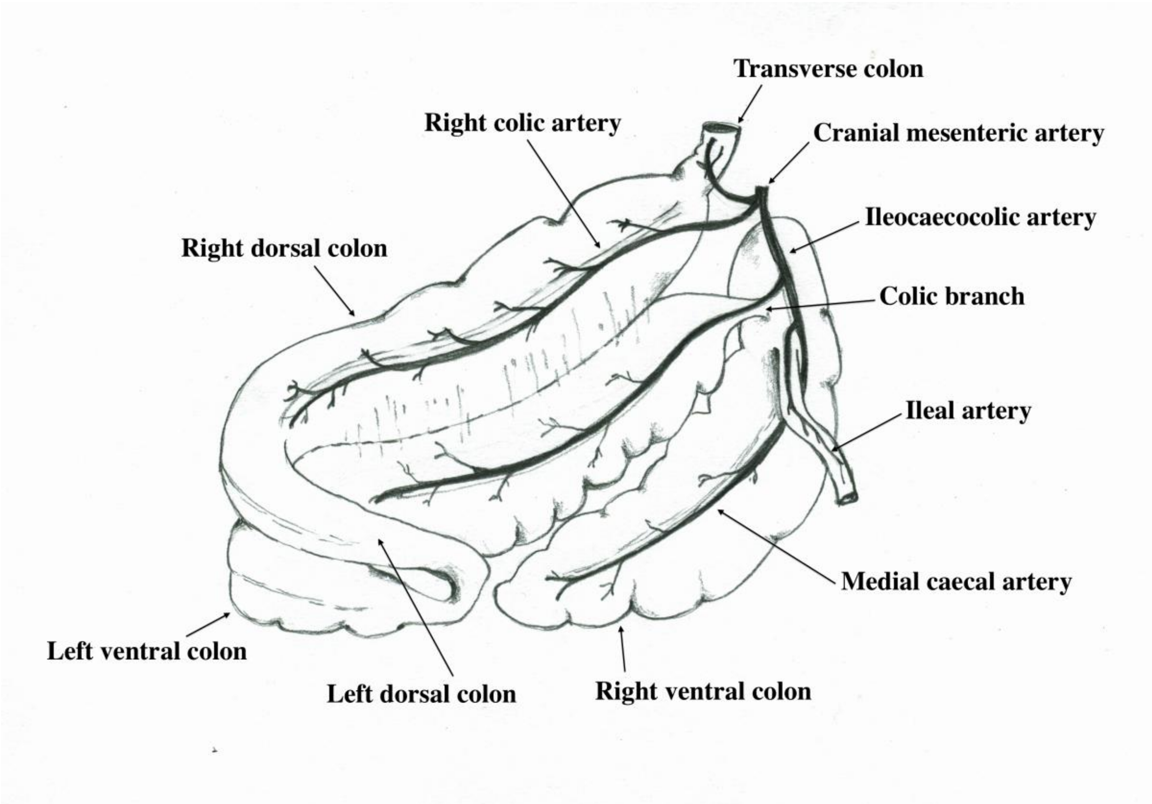


Figure 4: *A line drawing showing the vascular supply of the large colon.*

Arteries branch from the colonic vessels every 2cm and anastomose with oral and aboral vessels to form an anastomotic plexus or colonic rete, before continuing to the colonic tissue; here they penetrate the submucosa and form a submucosal vascular plexus [24]. The submucosal arterioles then branch to supply the mucosal capillary network [28]. The vascular supply to the large colon enters the colon through the caecum's attachment with the dorsal body wall, predisposing the colon and caecum to vascular compromise when LCV occurs [25]. The colonic rete may provide a collateral blood supply to the large colon, therefore reducing susceptibility of the colon to thromboembolic disease [29].

Physiology of the large colon

The major functions of the large intestine are microbial fermentation, mucosal absorption of short chain fatty acids (SCFAs), fluid and electrolyte absorption and the microbial synthesis of vitamins [30, 31, 32].

The horse is a hindgut fermenter, adapted to grazing on high fibre, low energy feed. The large intestine provides an anaerobic environment for the caecal and colonic microbiota, which consist of large numbers of bacteria, protozoa and fungi [33, 34]. Of these the dominant microbial population are the fibrolytic bacteria, which ferment slowly digestible carbohydrates, (for example cellulose and soluble fibre), to produce SCFAs, mainly acetate, propionate and butyrate [33, 35, 36, 37]. These SCFAs are readily absorbed and provide the major source of energy for the horse [30, 37]. Microbial synthesis and intestinal absorption of B-vitamins and vitamin K also occur in the large colon and provides the horses' entire requirement of these nutrients [33]. Absorption of the diverse population of microbes and microbial components, such as lipopolysacharide (LPS), into the horses' circulation is largely prevented by the mucosal barrier, which acts as an effective defence mechanism. The barrier function is maintained by tight junctions between epithelial cells and by their secretions [38]. Small amounts of LPS that enter the portal circulation are neutralised by circulating anti-LPS antibodies and cleared by the mononuclear phagocytic system of the liver [39].

The large intestine recovers 20% to 30% of a horse's body weight in water over a 24 hour period [30]. The secretion and absorption of water and electrolytes in the large colon maintains the correct osmolality and pH of the intestinal lumen for the microbial population and maintains the viscosity of the ingesta, assisting its aboral, progressive flow.

The flow of ingesta is also facilitated by the sigmoid flexure, at the caeco-colic junction, which prevents the reflux of ingesta from the colon into the caecum. In addition, no reflux occurs from the ventral to the dorsal colon [31, 33, 40]. Smooth muscle contractions result in the mixing and distal propulsion of ingesta. The direction and velocity of the propagation of these contractions are determined by "slow waves"; these are rhythmic oscillations in membrane potential [41]. The interstitial cells of Cajal (ICC) are the enteric pacemaker cells which are responsible for the initiation of slow wave activity, and facilitate the propagation of electrical events [42, 43, 44]. The slow aboral flow of ingesta facilitates the absorption of nutrients, by mixing ingesta and exposing nutrients to the mucosa for absorption [33].

Pathophysiology of large colon volvulus

Strangulating obstruction of the large colon can be correctly termed either volvulus or torsion; "Torsion" refers to rotation of the bowel about its long axis, whilst "volvulus" is defined as the rotation of a segment of bowel about the long axis of its mesentery [25]. For the purposes of consistency in this manuscript the term large colon volvulus (LCV) will be utilised. LCV arises either at the caecocolic mesentery, including the caecum, or more commonly, further distally at the caecocolic fold, not including the caecum. The volvulus is most often in a dorso-medial direction [8], which may be due to the caecocolic fold impeding dorso-lateral rotation of the ventral colon [10]. The right ventral colon rotates medially and dorsally (Fig. 5). Strangulating volvuli of 270 to 720 degrees have been described [8]. Rotation of the colon is possible due to the minimal anchoring attachments at the caecocolic fold and the mesocolic attachment to the root of the mesentery [25].

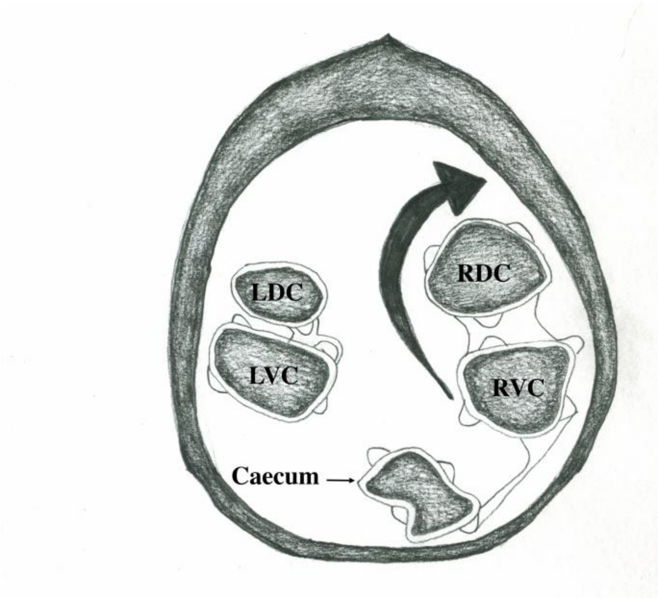


Figure 5: *A schematic diagram representing the more common dorso-medial direction of a LCV. The image is as seen from the tail-end of the horse.*

Physiological derangements of the large colon can result in alteration of the luminal environment, dysmotility, impaction, gaseous distension, and potentially large colon displacement or large colon volvulus [34, 37, 43, 45, 46]. Intestinal motility patterns are influenced by the interstitial cells of Cajal (ICC), the gastrointestinal pacemaker cells [42]. A reduction in the density of these cells may predispose to impactions, displacements or LCV; it has been reported that there is a significant reduction in the density of the ICC in pelvic flexure samples from horses with large colon disorders compared to control horses [43].

Alteration in diet affects the gastrointestinal microbiota and their metabolites, altering the luminal environment of the large colon. An increase in concentrate feed, and therefore an increase in hydrolysable and rapidly fermented carbohydrate, leads to an overgrowth of acidophilic *Streptococci* and *Lactobacilli*, an increased concentration of lactate, a reduction in the pH of the luminal environment and a subsequent decrease in the fibrolytic bacterial species [47, 48, 49, 50]. This has a negative impact on the health of the intestinal tissue. It has been demonstrated that in horses with simple colonic disruption and distension (SCOD) colic there is a relative abundance of *Streptococci* and *Lactobaccilli* and a decrease in the proportion of *Fibrobacter spp* [51]. The species that proliferate in a starch rich environment will not only produce excess lactate but also large amounts of CO_2 which is hypothesised to lead to distension and pain, and potentially displacement or volvulus of the large intestine [34].

LCV can be acute in onset, with sudden and complete rotation of the colon. However, in other instances volvulus can be more insidious in onset, secondary to displacement or impaction [24, 25]. Some horses will present with a history of mild colic signs, of over 24 hours duration, followed by a sudden increase in the degree of pain [24]. Impactions of the large colon most commonly occur at the pelvic flexure, or at the right dorsal colon, due to narrowing of the luminal diameter at both of these locations [3, 52]. Impaction of the colon will alter the mass and consistency of the material within the colon, and this can cause gaseous distension, disruption in the progressive motility, and ultimately displacement or rotation of the colon. The likelihood of a displacement or torsion occurring secondary to an impaction may be increased if the impaction consists of an accumulation of sand; over 30% of cases in three studies investigating horses with a sand impaction had a concurrent displacement or torsion [53, 54, 55]. This is presumed to be due to the weight and abrasiveness of sand causing intestinal distension and mucosal damage [24].

Strangulating LCV results in simultaneous occlusion of the intestinal lumen and occlusion of the large colon's vascular supply. The nature of this vascular occlusion can vary. In the majority of cases veins, due to their thinner, more compliant walls and lower hydrostatic pressure, are occluded prior to the arteries [56]. This is known as *haemorrhagic* *strangulating obstruction* and results not only in ischaemic injury, but also in severe congestion of the tissues, with intramural oedema, extravasation of red blood cells, microvascular thrombosis, mesothelial cell loss from the serosal surface and mucosal degeneration with loss of epithelium [38, 57, 58]. Alternatively, the venous and arterial supplies are occluded simultaneously, resulting in so-called *ischaemic strangulating obstruction,* which results in more rapid mucosal degeneration, but with reduced congestion of the tissues. This is less frequently noted in horses with large colon volvulus since the presence of ingesta within the intestinal lumen reduces the degree to which the intestine twists about its mesenteric axis [59]. Ischaemic injury also occurs when distension of the large colon causes increased intra-luminal pressure, which impedes micro-vascular perfusion [60, 61]. The degree of volvulus will directly influence the severity of vascular occlusion and rate of mucosal degeneration, and therefore will have a significant impact on a horse's pre-operative clinical parameters and prognosis for survival.

Intestinal mucosal epithelium is very susceptible to ischaemic injury due to the high energy requirement of the Na^+/K^+-ATPase that regulates ion and nutrient flux [38]. During the ischaemic conditions that occur following LCV cells undergo apoptosis rather than necrosis [62], with the more superficial epithelial cells being lost initially, followed by those cells in the crypts [57, 63]. Complete, irreversible mucosal degeneration develops within three hours of vascular occlusion [57]. Ischaemic injury can be exacerbated by injury that occurs during reperfusion after correction of the volvulus. Reperfusion injury is a result of the accumulated products that build up during ischaemia, and the reactive oxygen species that are generated on reperfusion [64, 65, 66]. However, the importance of reperfusion injury in LCV is questionable: recent experimental studies found complete ischemia of equine colonic mucosa for one hour, followed by reperfusion for 4 hours, did not result in functional or morphological evidence of reperfusion injury [64], and that equine colonic mucosa can repair during reperfusion [62, 67].

Breakdown of the mucosal barrier due to strangulating LCV causes the trans-mural and trans-vascular migration of microbes and pathogen associated molecular patterns, including LPS (endotoxin), bacterial lipoproteins and bacterial DNA, into the peritoneal cavity and circulation [68, 69, 70]. This results in the clinical manifestation of systemic inflammatory response syndrome (SIRS) [71]. The term SIRS has been proposed in the human literature to describe the clinical state resulting from an exaggerated and inappropriate systemic inflammatory response which can be initiated by infection, trauma, ischaemia, immune mediated disease, surgery, hypothermia or hyperthermia, or hypoxia [71, 72]. Pathogen associated molecular patterns in the circulation, such as LPS from gram-negative bacteria, are recognised by an extensive group of pattern recognition receptors (PRRs) on host cells. These PRRs include toll-like receptors, lectin receptors, retinoic acid inducible genel-like receptors, and nucleotide-binding oligomerisation domain (NOD)-like receptors [70]. Activation of PRRs results in the release of multiple endogenous molecular substances by host cells, each with a diverse array of biological activities. Molecules released include numerous cytokines, (such as tumour necrosis factor-alpha, interleukin 1, 4, 6, 8 and 10), eicosinoids, platelet activating factor, acute phase proteins, and reactive oxygen species [72]. The activation of this inflammatory cascade leads to pyrexia, neutrophil margination, (seen clinically as a neutropaenia), altered blood flow, increased vascular permeability, alteration in the balance between coagulation and fibrinolysis and immunosuppression. The subsequent systemic hypovolaemia and poor tissue perfusion causes tissue hypoxia and cardiovascular compromise, potentially resulting in multiple organ dysfunction and ultimately death [72, 73]. In cases of strangulating large colon volvulus this cardiovascular compromise is exacerbated by the distended large colon and caecum impeding venous return by compression of the vena cava [3] and by loss of fluid into the colon [74]. Respiratory compromise can occur due to excessive compression on the diaphragm by the distended large colon and caecum [24]. In addition damage of colonic vessels may result in thrombosis and segmental ischaemic necrosis post-operatively resulting in severe SIRS, cardiovascular compromise, clinical deterioration and death or a requirement for euthanasia [75].

In humans specific diagnostic criteria for SIRS have been proposed and scoring systems for SIRS are available offering prognostic information [71]. In humans in order for a diagnosis of SIRS to be made more than one of the following clinical manifestations must be present: pyrexia, tachycardia, tachypnoea or hyperventilation, or an alteration in white blood cell count or the presence of more than 10% band neutrophils [71]. There is currently no consensus on the diagnostic criteria for SIRS in horses, and similar prognostic scoring systems in horses with SIRS secondary to strangulating gastrointestinal disease are not available. Despite this the severity of an individuals systemic inflammatory response following strangulating large colon volvulus will markedly influence prognosis for survival.

Clinical presentation

Cases of LCV typically present, with acute, violent colic signs. Alternatively LCV may occur secondary to a displacement or impaction, with chronic, mild colic signs, followed by an acute exacerbation in the degree of pain [24, 25]. Clinical signs vary with the duration of strangulation, the degree of the distension of the large colon and the degree of cardiovascular compromise. Initially, despite severe abdominal pain, cardiovascular parameters will remain within normal limits and palpation per rectum may be unremarkable. As time elapses clinical signs of SIRS will become evident including tachycardia, tachypnoea, delayed capillary refill time, and red or purple mucus membranes. Abdominal distension is often evident, and distended, oedematous large colon can often be identified upon palpation per rectum [3]. Nasogastric intubation will occasionally yield reflux of gastric contents, which may be associated with tension on the duodenocolic ligament resulting in duodenal obstruction [8]. Abdominocentesis is rarely required to formulate a diagnosis and in cases of LCV the risk of enterocentesis is increased due to distension of the large colon.

Haematology and biochemistry may be normal initially in acute cases. As the disease progresses the packed cell volume will increase as a result of hypovolaemia. Due to loss of vascular integrity total plasma protein may fall, despite haemoconcentration, as plasma proteins leach into the lumen and walls of the large colon [25]. Elevation of plasma and peritoneal fluid lactate concentrations may be evident as a result of intestinal and mesenteric ischaemia and tissue hypoxia [76]. Increased thickness of the large colon wall on ultrasonography is an accurate and reliable pre-operative test to detect strangulating LCV [77, 78]. However, the severity of pain and obvious requirement for immediate surgical intervention often render elements of the clinical examination and diagnostic imaging unnecessary and impractical.

Treatment

Strangulating LCV requires rapid surgical intervention. Options for the surgical management of horses with a large colon volvulus include correction of the volvulus and replacement of the large colon, with or without colopexy to prevent recurrence, resection and anastomosis of the large colon (LCRA) or euthanasia on the operating table.

Prior to surgical intervention, resuscitation of circulating blood volume may be initiated with hypertonic saline and colloids. A ventral midline incision is made with the horse in dorsal recumbency. It is often necessary to extend the incision cranially to allow exteriorisation of the distended, oedematous colon. The caecum and colon may require decompression in situ, to improve venous return and ventilation, and to facilitate intestinal manipulation and abdominal exploration. LCV frequently results in oedema and increased friability of the large colon and care must be taken during exteriorisation to prevent inadvertent rupture, particularly of the right dorsal colon [24]. Evacuation of the large colon via a pelvic flexure enterotomy (Fig.6) can be performed prior to correction of the volvulus to reduce the weight of the colon and potentially to reduce the systemic absorption of microbial molecules (such as lipopolysacharide) [25].

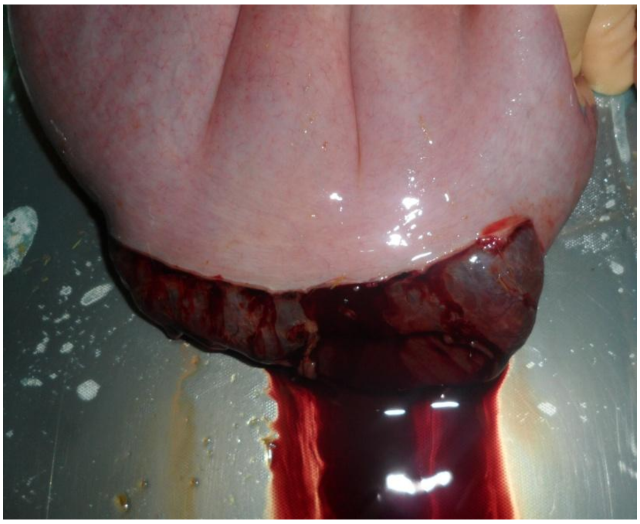


Figure 6: *Intra-operative photographic image of the site of a pelvic flexure enterotomy. The mucosa is congested and oedematous and haemorrhage from the site is present but reduced in quantity. The colonic serosa is slightly purple in colour.*

The serosal and mucosal colour, the degree of colon wall and mesocolon oedema, the presence of haemorrhage at the enterotomy site, and the presence of a palpable pulse should be evaluated. These intraoperative findings, in combination with the pre-operative parameters should be considered and a decision made to correct the volvulus and recover the horse, perform a LCRA, or to euthanase the horse on the operating table. Correction of the volvulus should be confirmed by assessing the normal position of the caecocolic ligament (Fig. 7) and by palpation of the straight mesenteric attachment of the right dorsal colon to the dorsal body wall.

Figure 7: *Intra-operative photographic image showing the caecocolic ligament in a normal orientation.*

Large colon resection and anastomosis (LCRA) allows resection of devitalised tissue and prevents recurrence of the volvulus. Techniques for resection of the large colon include resection and end to end anastomosis, and resection and side to side anastomosis [79, 80, 81, 82, 83]. The decision to perform a resection and anastomosis will be based on the pre and intra-operative findings, surgeon preference and the location of the volvulus. If the line of devitalised tissue is distal to the caeco-colic ligament it is possible to resect the entire section of affected bowel and the anastomosis is not performed in compromised tissue [24]. Colopexy is usually reserved for broodmares that have had a previous LCV or large colon displacement to prevent recurrence [84]. It is unsuitable in horses that undertake high impact exercise [85] or in cases with severe vascular compromise of the large colon [86].

Horses require intensive post-operative management following surgical correction of LCV, due to the potential for severe systemic inflammatory response and loss of intravascular oncotic pressure. Careful monitoring of the horses' demeanour, clinical parameters, haematocrit and total protein is required, to assess progress and determine appropriate post-operative therapy. Aggressive fluid therapy, with intravenous crystalloids, synthetic colloids or plasma, is often required in an attempt to correct hypovolaemia and increase oncotic pressure [75]. Flunixin meglumine is a non-steroidal anti-inflammatory drug often utilised in the post-operative period, that ameliorates the clinical signs associated with systemic inflammatory response syndrome and has been shown to increase the time until death in fatal models of equine endotoxaemia [87, 88, 89, 90]. Lidocaine has also been shown to decrease the severity of clinical signs in experimentally induced equine endotoxaemia and has been shown to provide effective analgesia and to inhibit leukocyte migration [91, 92, 93]. Polymixin B is a cationic antimicrobial, that has a high affinity for the lipid A portion of endotoxin and forms a stable complex on binding to endotoxin, preventing its binding to cell receptors [39]. However, there is limited evidence that it has a significant effect in vivo, when administered following an endotoxic insult [94], and its use following LCV is therefore debatable. Other therapies that have been proposed to benefit the endotoxic horse following LCV include dimethyl sulfoxide, pentoxyfylline, heparin and ethyl pyruvate [39, 75, 95, 96].

Despite aggressive and rapid surgical management and intensive post-operative treatment LCV is often associated with post-operative morbidity and mortality.

Post-operative morbidity

Post-operative morbidity in horses with a LCV is partially related to the degree of the systemic inflammatory response and includes pyrexia, jugular thrombophlebitis and hypovolaemia, colic, diarrhoea, peritonitis, ileus, incisional infection and incisional herniation [97]. Postoperative morbidity compromises patient welfare, increases the cost of treatment and is distressing for the owner [7, 98]. The risk of post-operative colic is significantly increased in horses with LCV [7]. This may be due to adhesion formation or stenosis at localised sites of ischaemia that have repaired post-operatively [7]. Alternatively abnormal intestinal physiology may predispose certain individuals to recurrent LCV or large colon displacement [43].

Post-operative survival

Knowledge of post-operative survival patterns and identification of factors that are significantly associated with prognosis, following surgical treatment of colic, allows clinicians and owners to make evidence based decisions regarding the management of individual horses [99, 100]. It has been recognised that key prognostic indicators, and survival patterns following surgery, differ for various types of colic [6, 99, 100].

Survival to hospital discharge following LCV is reported to lie between 35-74% [8, 9, 10, 11]. This wide range can partially be attributed to differences between hospital populations, a lack of consistency in the definition of a strangulating LCV and progress made over time with surgical techniques and post-operative management. Although volvulus greater than or equal to 360 degrees has been found to be significantly associated with poor long-term survival [6], relatively few studies provide information regarding long-term survival patterns following hospital discharge in horses with LCV. In addition, specific determinants of survival for horses with a strangulating LCV have not been investigated in a large number of cases using survival analysis.

Elevated heart rate and PCV on admission are significantly associated with post-operative mortality in horses undergoing surgery for large intestinal disease [6] and a pre-operative

PCV greater than 50% has been found to correlate with a non-viable colon [79]. Pre-operative plasma lactate has also been utilised as a predictor for colonic viability and survival; in one study horses with a plasma lactate concentration greater than 7.0mmol/L had a less than 30% chance of survival [76].

Visual assessment of the viability of the large colon at exploratory laparotomy is challenging and often unreliable [11]. Intra-operative measurement of colonic luminal pressure, although inexpensive and practical is not an accurate prognostic indicator [101]. Other methods of assessment of viability, that are often unreliable or not readily available, include pulse or surface oximetry, and fluorometric evaluation [102, 103]. Histopathologic evaluation of an intra-operative biopsy, collected from the pelvic flexure, has previously been shown to be a highly sensitive and specific predictor of post-operative survival in cases of LCV [104, 105]. Features found to accurately predict survival included the percentage of glandular epithelium lost or separated from the basement membrane, and the interstitial crypt ratio, which was defined as the ratio of glandular crypts and interstitium within the lamina propria [104]. However, intra-operative evaluation of a biopsy is impractical and a more recent studies have found histopathological evaluation of pelvic flexure biopsies did not accurately predict survival, while certain clinical parameters did [106, 107].

It remains unclear as to whether performing large colon resection and anastomosis (LCRA) increases prognosis for survival. In one hospital population LCRA was found to be associated with an increased risk of post-operative mortality [6]; however, another centre has reported survival to three years post-operatively to be over 70% when LCRA was performed in horses with LCV [11], which is greater than survival rates reported from other centres where LCRA was not performed.

Post-operative persistent tachycardia, with a heart rate of over 80bpm, or a total protein of less than 40 g/litre, have been reported to indicate a poor prognosis for survival [75]. In horses undergoing LCRA, heart rate 24 hours post-operatively was significantly associated with post-operative mortality. Sheats *et al.* [108] suggested that ultrasonographic assessment of the rate of involution of the colon wall could be utilised to predict morbidity and mortality in cases of LCV; however, the study did not demonstrate a significant difference between survivors and non-survivors.

Specific determinants of survival and the pattern of long-term survival in horses with a strangulating LCV have not been investigated in a large number of cases using survival analysis. Knowledge of risk factors for survival in horse with LCV could assist clinicians with decision-making in the management of theses cases. Clinicians could also utilise the information to provide owners of these horses with evidence-based information regarding their prognosis for survival.

Epidemiology

The reported incidence of colic in the horse population is between 3.5 to 10.6 colic episodes per 100 horses per year [1, 2, 12, 17, 109]. Of these colic episodes, reports estimate 7% to 17% are surgical in nature.

Various epidemiological studies have shown that colic, like many non-communicable diseases is complex and multifactorial in nature [110]. These studies have identified risk factors associated with colic in general [1, 12, 16, 17, 18, 111, 112], and risk factors associated with specific types of colic. Specific types of colic that have been investigated in epidemiological studies include strangulation of small intestine by pedunculated lipomas

[113, 114], ileal impactions [21, 115], simple colonic obstruction/ distension colic (SCOD) [20], impaction colic [116, 117] and epiploic foramen entrapment [13, 14]. There are currently no published reports investigating specific risk factors for LCV.

Studies identifying risk factors for colic in general are valuable, since the underlying gastrointestinal dysfunction often remains unknown [118]. However, risk factors vary for various types of colic, and the identification of more specific risk factors can further our understanding of disease causality, can be used to identify high-risk individuals and can assist with diagnostic investigations. Identification of modifiable risk factors for either colic in general, or for specific types of colic, can enable the development of disease prevention strategies that may reduce the incidence of colic.

Various study designs have been utilised to investigate risk factors for colic in general and for specific types of colic, including cohort [1, 17, 18], cross-sectional [109] and case-control studies [13, 14, 19, 20, 21, 111, 112, 117, 119]. Case-control studies are an efficient method for the investigation of relatively uncommon diseases [120], making them suitable for identifying risk factors for specific types of colic, such as LCV.

Horse-level risk factors

Horse-level risk factors may increase or decrease the likelihood of an individual from suffering from colic [118]. Whilst these are unlikely to be modifiable, knowledge of these factors may identify areas for future research, assist with the recognition of high-risk individuals, and be of use in diagnostic investigations [118].

Gender

Certain types of colic, such as inguinal herniation in stallions or uterine torsion in mares, are gender specific, but in the majority of studies there is no clear association between gender and colic [12]. Associations between gender and colic may be confounded by use of the horse, or other management practices [12]. Clinical experience suggests that mares in the post-partum period are at increased risk of suffering from a LCV [24, 121]. This is supported by work by Kaneene *et al.* [17] who found that foaling was significantly associated with an increased risk of colic.

Age

Variations in study population, case definition and study design may explain the conflicting results of studies that have previously investigated associations between age and colic. One study identified foals of <6 months to be at reduced risk of colic [1], although ascarid impactions and intussusceptions are reported to be more prevalent in horses of this age [122, 123]. Tinker *et al.* [18] identified horses between 2 and 10 years to be at increased risk of colic, whereas horses of increasing age, horses >8 years, and horses >10 years, were found to be at increased risk in other studies [17, 111, 119]. Older horses and ponies have been consistently recognised to be at increased risk of suffering from colic due to a pedunculated lipoma [113, 114, 124] and increasing age was found to be associated with impaction colic in donkeys in the UK [116]. Younger horses have been found to have an increased risk of equine grass sickness [125, 126, 127, 128].

Breed

The association between breed of horse and colic varies between studies [12]. A number of studies have identified Arabs to be at increased risk of suffering from colic [19, 111, 119], although Tinker *et al.* identified Arab horses to be at reduced risk [2]. Thoroughbreds have been found to be at increased risk of colic in a number of studies[1, 2]. Other studies have identified no association between breed and colic [17]. Clinical experience suggests that

Thoroughbred broodmares are at increased risk of LCV and there is anecdotal evidence that that there is increased incidence of large colon displacement or torsion in Warmblood breeds [121].

Previous history of colic

Horses with a history of colic are at increased risk of suffering further colic episodes [1, 12, 15, 18, 119]. Specific to colic associated with the large colon, Hillyer *et al.* (2002) [20] found that there was increased risk of simple colonic obstruction and distension (SCOD) in those horses with a previous history colic, and donkeys with a history of colic were found to have an increased risk of a large colon impaction [116]. Previous exploratory laparotomy is a known risk factor for colic [111, 119] and the risk of post-operative colic is significantly increased in horses that have previously had a LCV [7]. In one study of horses undergoing large colon resection and anastomoses over 20% of the study population had had a large intestinal disorder identified at an earlier exploratory laparotomy [129]. Therefore one could hypothesise that previous exploratory laparotomy is a risk factor for LCV.

Behavioural abnormalities

Horses that exhibit crib-biting / windsucking behaviour have been identified to be at increased risk of recurrent colic [15] and of epiploic foramen entrapment [13, 14] Crib-biting and wind-sucking behaviour was also found to be very strongly associated with an increased risk of SCOD (Odds Ratio [OR] 89.46, 95% confidence interval [CI] 8.98 – 890.69) [20]. Therefore it could be hypothesised that these behaviours may also be associated with increased risk of LCV. McGreevy *et al.* (2001) found horses that displayed crib-biting or wind-sucking behaviour have significantly longer total gut transit times than other horses, suggesting that these behaviours may occur secondarily to some form of gastrointestinal malfunction. Alternatively crib-biting/wind-sucking behaviour may be a marker for management variables such as access to turnout and diet [15].

Management-level risk factors

It is likely that the seasonal incidence of colic is associated with alterable management factors common to the time of year [130]. Hillyer *et al.* [109] reported a seasonal pattern of colic in the UK but with a significant difference between National Hunt and Flat Racing premises, with an increased incidence of colic during periods of increased activity. The incidence of large colon displacements and torsions has been shown to peak in the spring and autumn, with a six and twelve month cyclical pattern identified [130].

Nutrition and feeding practices

Numerous studies have identified various associations between feed types and feeding practices with colic. It is unsurprising, given the effect of increasing starch intake on the microbial population of the large colon, that feeding a greater amount of concentrate feed is associated with an increased risk of colic [1, 18, 112, 119]. Feeding more than 2.7kg oats/day was significantly associated with colic in a study by Hudson *et al.* (2001) [112] and Tinker *et al.* (1997) [18] found increasing concentrate intake was associated with an increasing risk of colic. Specific to the large colon, feeding a concentrate ration significantly increased the risk of large colon impaction in donkeys [117].

The association between colic and feeding particular types of concentrate is unclear; two studies identified no association between type of concentrate and colic [1, 119], whilst another study identified that processed concentrates, such as pellets or sweet feeds increased the risk [18]. A recent change in the amount of concentrate fed was significantly associated with increased risk of colic [18], and with an increased risk of SCOD [20]. Hudson *et al.* (2001) [112] found a recent change in the type of concentrate fed was also associated with an increased risk of colic.

Hudson *et al*. (2001) [112] also reported that feeding hay from round bales and feeding hay other than alfalfa, coastal, or Bermuda hay, increased the risk of colic. However, in another study horses fed Bermuda grass hay were significantly more likely to have had an episode of colic, or to suffer from recurrent colic [111]. Feeding of Bermuda grass hay was also identified as a risk factor for ileal impactions in the USA [115]. A significant association was identified in another study between the feeding of alfalfa hay and enterolithiasis [131]. Hudson *et al.* (2001) [112] and Cohen *et al*. (1999) [119] both identified a recent change in the type or batch of hay to be associated with increased risk of colic, and Tinker *et al.* (1997) [18] found more than the expected one change/year of hay to be associated with increased risk.

Stabling and turnout

Stabling or reduced time at pasture has been reported as a risk factor for colic in numerous studies [112, 132], including large colon disorders [20, 117] and large intestinal motility is reduced in stabled horses compared to horses kept at grass [133]. It should be noted that increase in stabling might be a marker for other confounding variables such as increased supplementary feeding of forage and reduction in exercise. In addition mild episodes of colic may not be observed in horses kept at pasture [17]. Traub-Dargatz *et al.* [1] did not identify an association between colic and type of pasture, pasture quality, or stocking density. In contrast, another study found a stocking density of <0.5 horses/acre to be significantly associated with an increased likelihood of colic [111].

Access to water

Horses with access to water sources other than buckets, troughs or tanks were found to be at decreased risk of suffering from colic in one study [17]. This was in agreement with the findings of Cohen *et al*. (1995), who found that horses with access to a pond were at decreased risk of colic. A study conducted by Reeves *et al.* identified horses without access to water in outdoor enclosures were at increased risk of colic [16]. Cox *et al.* (2009) [117] also found that donkeys without water sources in their outdoor enclosure were at increased risk of an impaction.

Exercise

Hillyer *et al.* [109] reported an increased incidence of colic during the stages of training with increased levels of activity in National Hunt and Flat racing premises; however, this study did not take into account other confounding factors such as nutrition or stabling. A recent change in exercise was associated with an increased likelihood of SCOD colic when taking these confounding variables into account in the final multivariable model. This increased risk was greatest when the change in exercise had occurred in the previous week [20].

Transport

There are conflicting reports on the association between transportation and colic. White (1997) [134] reported an increased risk of colic following transport. In contrast Cohen *et al.* (1995) found no association between colic and transportation. A history of transport in the proceeding 24 hours was associated with a large increase in the risk of SCOD colic (OR 17.48, 95% CI 2.16 – 141.35) [20]. This finding may be related to the act of transportation itself or it may be confounded by the large number of other management changes that occur simultaneously, such as change in premises, physical constraint, water or feed deprivation, which were either not significant when assessed alone, or could not be measured [12].

Parasites and anthelmintic administration

Parasites are a well documented cause of colic in the horse. In the past *Strongylus vulgaris* was alleged to cause up to 90% of colic episodes [12]. The availability of modern anthelmintics has meant reports of colic secondary to *S. vulgaris* infection are now uncommon. The tapeworm *Anoplocephala perfoliata* has been shown to be significantly associated with ileal impaction and spasmodic colic [21, 115] and clinical or pathological evidence of concurrent larval cyathostomiasis is reported with caecocaecal and caecocolic intussusceptions [135].

Some studies have identified no association of colic with a parasite control programme. However, Cohen *et al.* (1999) [119] found an increased risk of colic in the immediate period following anthelmintic administration; this may occur in horses with a large parasite burden, where anthelmintic treatment results in the death of large numbers of parasites, and subsequent intestinal inflammation. Strategies that prevent the accumulation of large numbers of parasites appear to reduce the risk of colic. Uhlinger (1990) [136] conducted an intervention study over a 5 year period and reported the greatest reduction in the incidence of colic with use of the most efficacious anthelmintic schedule. A number of other studies also report a decreased risk of colic associated with anthelmintic use [16, 112]. Absence of moxidectin or ivermectin anthelmintic treatment in the previous 12 months was associated with SCOD colic [20]. It is unclear whether anthelmintic treatment and parasite burden are risk factors for LCV.

Dental pathology and dental prophylaxis

A number of dental pathologies, including worn teeth, missing teeth, ulcers and diastemata have been found to be associated with impaction colic in donkeys [116, 117]. Dental abnormalities were associated with an increased likelihood of recurrent colic in the horse [15]. Hillyer *et al.* (2002) [20] found that horses that had their teeth checked or treated fewer times/year were at increased risk of SCOD.

Vaccination

Recent vaccination was found to increase the risk of impaction colic in donkeys [117] and, in the USA, recent vaccination for Potomac horse fever increased the risk of colic in horses [18]. This is difficult to explain, but may be due to change in routine at the time of vaccination, or due to a systemic response to the vaccine.

Premises / owner factors

An increasing number of carers was found to increase the likelihood of large colon impaction in donkeys [117]. It has also been shown that horses whose owners provide their care are at decreased risk of colic compared to those horses cared for by a non-owner [16, 109]. This may be because owners provide more consistent and dedicated care for their horses, or may be due to other confounding factors, such as the number of horses on premises, or the horse's exercise regime.

Use of horse

Some studies found that horses used for showing, eventing or horses in training, particularly flat race horses, were at increased risk of colic [17, 18, 109]; however, various confounding factors, such as nutrition, stabling and exercise were not taken into account in these studies.

Clinical experience suggests that mares in the post-partum period are at increased risk of suffering from a LCV [24, 121] and this will be one of the hypotheses we will be testing in this thesis.

In conclusion, various factors have been identified as risk factors for colic and for specific types of colic. Whilst some are consistently reported, other factors vary between studies. These contradictory results may be due to variations in the aetiology of different types of colic, and variations in study populations, study design, and methods of analysis. No study has been undertaken previously to investigate specific risk factors for LCV.

Thesis aims and outline

The studies presented in this thesis aimed to investigate the epidemiology of strangulating LCV in the horse, with three primary objectives:

1) To describe the long-term pattern of survival in horses with a strangulating LCV.
2) To identify risk factors for survival in horses with a strangulating LCV.
3) To identify horse and management level risk factors for strangulating LCV.

To achieve these objectives two epidemiological studies were undertaken in the UK.

Chapter Two reports the findings of a retrospective study investigating survival of horses following strangulating LCV. Mortality rates, causes of death or euthanasia are described and Cox proportional hazards models were used to investigate factors associated with mortality in horses with a strangulating LCV undergoing anaesthesia and exploratory laparotomy at a UK equine hospital over a 10 year period.

Chapter Three presents the results of a prospective, multi-centre, case-control study. Cases of strangulating LCV were recruited from 4 clinics in the UK and controls were randomly selected from the client population of these clinics. Multivariable logistic regression was used to investigate horse and management level variables associated with increased risk of strangulating LCV.

Chapter Four summarises the information gained from these studies, discusses the practical applications for these findings and highlights areas that merit further research.

CHAPTER 2

Survival following strangulating large colon volvulus

Work presented in this chapter has been accepted for publication during the writing of this thesis (see General Appendix):

Suthers J.M., Pinchbeck G.L., Proudman C.J., Archer D.C. (2013). Survival of horses following strangulating large colon volvulus. *Equine Veterinary Journal,* **45** (22), 19-223.

Abstract

The pattern of long-term survival and specific factors associated with long-term survival have not previously been evaluated in horses with a strangulating large colon volvulus (LCV). The aims of this study were to provide data on the long-term survival of horses with LCV and to identify pre-, intra- and post-operative variables associated with survival.

Clinical data was extracted retrospectively from hospital records for horses with a strangulating LCV (≥ 360˚) undergoing general anaesthesia. Long-term follow up information was obtained from owners via a telephone questionnaire. Two multivariable Cox proportional hazards models for post-operative survival time were developed: model one included all horses and evaluated pre-operative variables and model two included horses that survived anaesthesia and evaluated pre, intra and post-operative variables.

The study population comprised 116 horses. Eighty-nine horses (76.6%) survived general anaesthesia. Of these, the percentage that survived until discharge, to one year, and to two years was 70.7%, 48.3% and 33.7% respectively. Median survival time for horses that survived general anaesthesia was 365 days. In model one, increased pre-operative packed cell volume (PCV) was significantly associated with reduced post-operative survival (Hazard Ratio [HR] 1.08, 95% confidence intervals [CI] 1.05 – 1.11). However, this effect changed over time. In model two, abnormal serosal colour intra-operatively (HR 3.61, 95% CI 1.55 – 8.44), increased heart rate at 48 hours post-surgery (HR 1.04, 95% CI 1.02 – 1.06), and colic during post-operative hospitalisation (HR 2.63, 95% CI 1.00 – 6.95), were all significantly associated with reduced post-operative survival.

Survival time in horses with a LCV was associated with pre-operative PCV, serosal colour, heart rate at 48 hours post-operatively, and colic during post-operative hospitalisation. This study provides evidence based information on the long-term survival of horses with LCV and identifies parameters that may assist decision-making by clinicians and owners.

Introduction

Identification of factors that are significantly associated with prognosis following surgical treatment of colic allows clinicians and owners to make informed decisions regarding the management of individual horses [99, 100]. It has been recognised that these key prognostic indicators, and survival patterns following surgery, differ for various types of colic [6, 99, 100]. However, relatively few studies provide information regarding survival patterns following hospital discharge or on key prognostic indicators for specific surgical lesions.

Large colon volvulus (LCV) is one of the most painful and rapidly fatal causes of colic in the horse [3]. In some hospital populations LCV represents between 10 and 20% of horses with colic that undergo exploratory laparotomy [4, 5]. Volvulus greater than or equal to 360 degrees has been found to be strongly associated with poor survival [6], with survival to hospital discharge following strangulating LCV reported to lie between 36 and 74% [9, 10, 11]. It has been previously identified that age, pre-operative PCV and heart rate are significantly associated with post-operative mortality in horses with colic due to large intestinal disease; however, horses with a strangulating LCV only represented 20% of the cases in this study [6]. Post-operative persistent tachycardia has been previously suggested to be a poor prognostic indicator for survival in horses with LCV [75] In horses undergoing large colon resection and anastomosis (LCRA), heart rate at 24 hours post-operatively was significantly associated with post-operative mortality [129]. However, specific determinants of survival for horses with a strangulating LCV have not been investigated in a large number of cases using survival analysis. The aims of this study were to provide data on the long-term survival of horses with strangulating LCV and to identify pre, intra and post-operative variables associated with survival.

Materials and methods

Study population

The case records of all horses with a LCV, (greater than or equal to 360 degrees), identified at exploratory laparotomy between 1st January 2001 and 31st December 2010 at the Philip Leverhulme Equine Hospital, United Kingdom were reviewed. Horses were included if they underwent general anaesthesia and exploratory laparotomy. Horses that were euthanased during surgery were included, but horses that died or were euthanased prior to anaesthesia would not have had an accurate diagnosis, and therefore were not included in the study population.

Pre-, intra- and post-operative data were extracted retrospectively from hospital records and entered into a computer database. Short-term survival data whilst in the hospital (reason for death and date of death) were obtained from hospital records. To evaluate long term survival, telephone questionnaires with owners were conducted following discharge from the hospital. The telephone questionnaire asked the date of death and reason for death. Questionnaires were conducted quarterly for the first year following colic surgery and bi-annually thereafter as part of an on going study on colic survival [6]. This was with the exception of a 30-month period during which questionnaires were suspended due to an alteration in the study's funding. Owners were re-contacted after this period. All horses remained in the study until they died or were lost to follow up, for example, following a change of ownership. In cases where the owner was unable to provide the exact date of death or censorship, this was defined as the mid-point of the week or month in which the horse was reported to have died or been sold. In this study the term death includes death due to euthanasia. Data collection, was primarily conducted by the author of this thesis, but utilised a pre-existing colic database, which contained some of the required pre-, intra- and post-operative data. This database was funded by The Horse Trust and the Petplan Charitable Trust, with data collection carried out by clerical staff, Jan Smith and Jane Barnes.

Statistical analysis

Descriptive data were generated and survival time was used to construct a Kaplan-Meier plot of cumulative probability of survival [137]. The study population was divided into two groups. Group one included the entire study population and evaluated pre-operative variables (model one). Group two included only those horses that recovered successfully from anaesthesia and evaluated pre, intra and post-operative variables (model two). Prior to univariable analysis, all variables were assessed for correlation using Spearman's rank correlation coefficients. Where Spearman's rank correlation coefficient was >0.8, the most statistically significant or biologically plausible variable was selected.

For both groups association with survival time was modelled using Cox proportional hazards models [137]. Survival time (days) was measured as a continuous variable starting from the date of induction of anaesthesia until death or censoring. Potential explanatory variables were screened for univariable association with survival time. The functional form of the relationship between continuous variables and survival time was modelled using penalised Cox models and by generating smoothing splines for each continuous variable [138]. Variables showing some evidence of univariable association with outcome ($P<0.2$) were evaluated in a multivariable Cox proportional hazards model, which was constructed using a backwards, stepwise elimination procedure [137]. Variables remained in the model if they significantly improved the fit ($p\leq0.05$) assessed using the likelihood ratio statistic. Variables with >33% of missing values were excluded from the initial model-building procedure. Biologically plausible interaction terms for variables remaining in the final model were assessed. As Cox models assume proportional hazards, (i.e. the effect of a variable on the outcome is constant over time), model diagnostics performed included Schoenfield residual plots to assess proportionality throughout survival time. In addition, graphical assessment of log cumulative hazards plots for categorical variables was performed. Scaled changes in the regression coefficient for each observation (delta betas) were used to evaluate potential leverage by individual observations for each variable [137]. The model was rerun excluding observations with large delta-beta values (>0.4 or <-0.4) to evaluate their influence on parameter estimates. The critical probability for all analyses was set at 0.05.

Results

The study population included 116 horses, of which 63 (54%) were mares, 52 (45%) were geldings and 1(1%) was a stallion. The median age of horses was 11 years (range 1-28 years) and the median weight was 580kg (range 350-750kg). The median duration of colic signs prior to surgery was 10 hours (range 3-96 hours). Median heart rate on admission was 60 beats/min (range 32-108 beats/min) and median packed cell volume (PCV) was 45% (range 27-65%). Only two horses underwent a large intestinal resection.

Of the 116 horses, 89 (76.7%) recovered from anaesthesia. Of these horses, the percentage that survived until discharge, to one year, and to two years was 70.8%, 48.3% and 33.7% respectively. The reasons for death are shown in table 1.

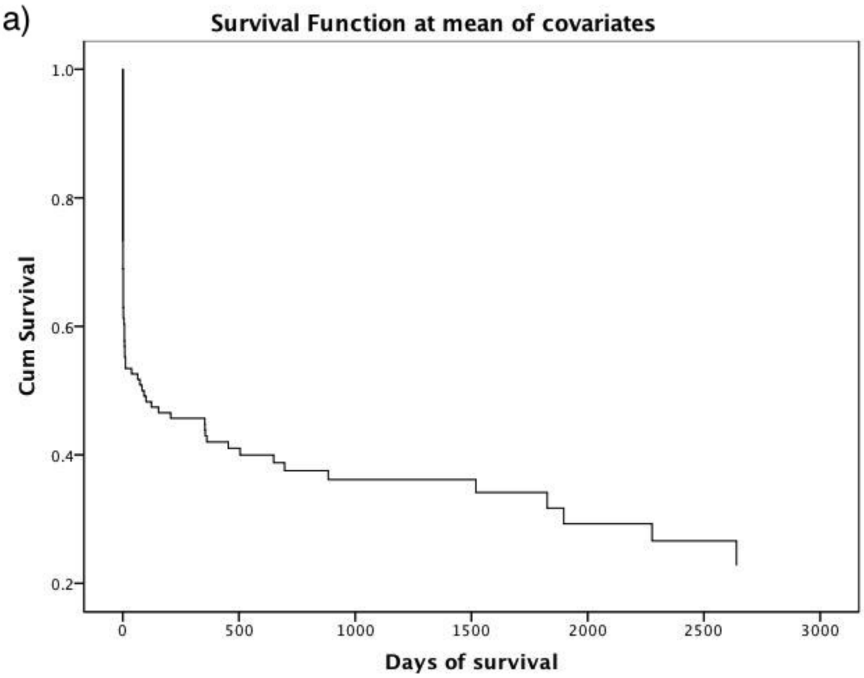
Time post-operatively	Reason for death or euthanasia	Number of horses	% of deaths
During surgery (n=23)	Euthanased: non viable colon[1]	18	24
	Euthanased: unable to correct torsion	3	4
	Euthanased: second LCV	1	1.3
	Died under anaesthesia	1	1.3
During recovery from anaesthesia (n=4)	Euthanased: Unable to stand	2	2.7
	Died: Unable to stand	2	2.7
During hospitalisation (n=26)	Euthanased: Systemic inflammatory response syndrome (SIRS) / pain / colon necrosis	23	30.7
	Euthanased: gastric rupture	2	2.7
	Euthanased: concurrent synovial sepsis	1	1.3
Following hospital discharge (n=22)	Colic	17	22.7
	Non-colic related	3	4
	Unknown	2	2.7

Table 1: Reasons *for death or euthanasia during intra and post-operative period.*

[1] Non-viable large colon as defined by surgeon at the time of exploratory laparotomy and recorded in the surgical report.

Sixteen horses were lost to follow up. The study included 67,386 days of survival. Median survival time for all horses was 88 days (Fig.1a) and median survival time for those horses that successfully recovered from anaesthesia was 365 days (Fig.1b).

a)



b)

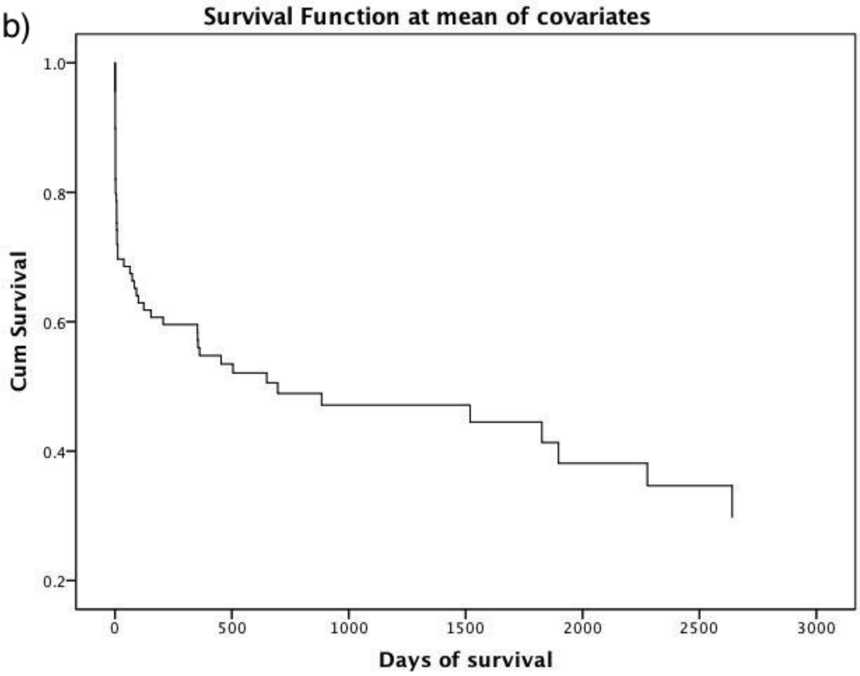


Figure 1a and Figure 1b: *Kaplan-Meier survival curves for a) 116 horses undergoing general anaesthesia and exploratory laparotomy for LCV and b) 89 horses recovering from anaesthesia following exploratory laparotomy for LCV.*

The results of univariable relationships of potential explanatory pre-operative variables with survival time as the outcome measure, when all the horses were included (Group one) are shown in the appendix to Chapter 2. On univariable analysis pre-operative variables significantly associated with increased risk of post-operative death were the degree of pain on admission, pre-operative heart rate and pre-operative PCV. The final Cox proportional hazards model (Model one) included only the variable pre-operative PCV, which was positively and significantly associated with the risk of post-operative death and demonstrated a linearly increasing likelihood of mortality (Fig. 2 and Table 2).

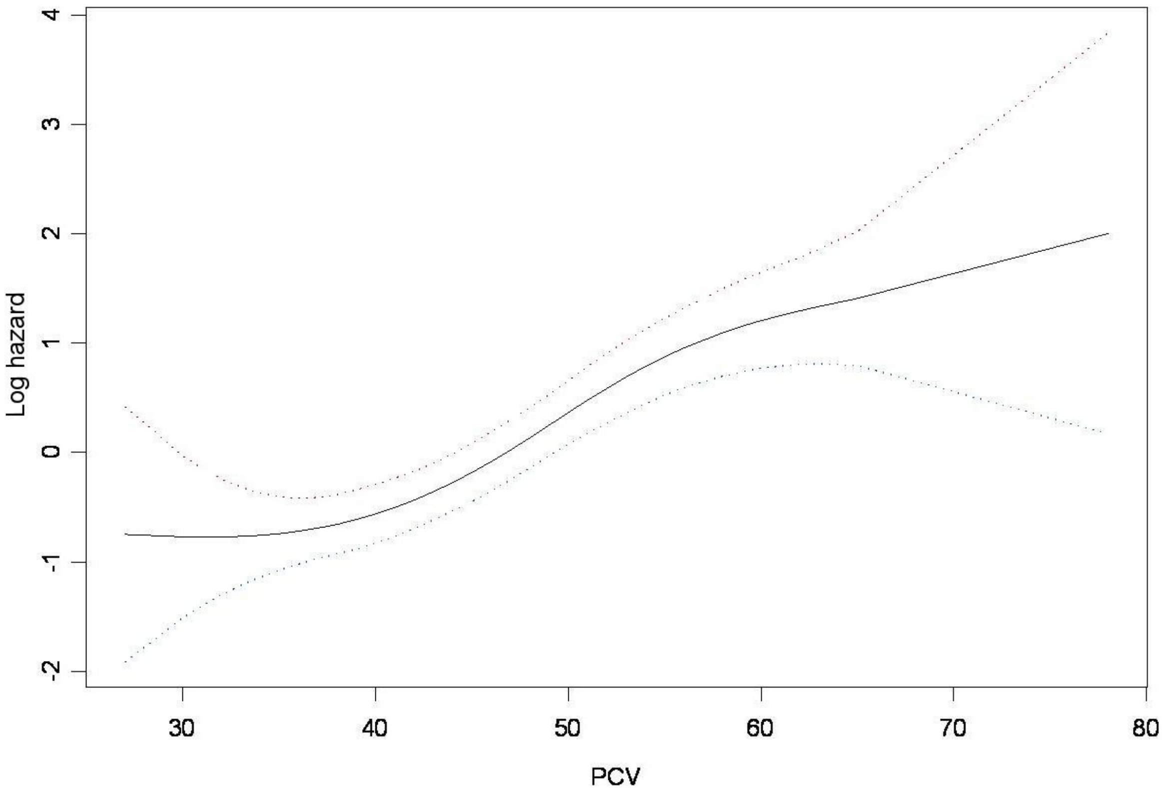


Figure 2: *Graph illustrating the shape of the relationship between PCV on admission and the risk of mortality in 116 horses undergoing surgery for LCV. Spline fit (solid line), with 95% confidence intervals (dotted lines) from univariable penalised Cox proportional hazards regression model are illustrated.*

Variable	**Coefficient**	**Standard error**	**Hazard ratio**	**95% Confidence Intervals**	**p-value**
PCV on admission (%)	0.08	0.02	1.08	1.05-1.11	<0.001
PCV ln(time) interaction effect	-0.04	0.01	0.96	0.95-0.97	<0.001

Table 2: *Final, multivariable Cox proportional hazards model (Model one) for post-operative death in 116 horses undergoing anaesthesia and exploratory laparotomy for LCV, including a PCV*ln(time) interaction term.*

However, there was evidence of significant non-proportionality of the effect of the variable PCV over time (Fig. 3), with reduced risk over time (p<0.007). A PCV*ln(time) interaction term was added to the model (Table 2). The effect of PCV was allowed to interact with time on a natural log scale; this was chosen since it was assumed that the effect of PCV would be greatest in the immediate post-operative period and then reduce as time passed. The PCV*ln(time) interaction term was significant, confirming that the effect of PCV does vary with time. Using this model, the hazard ratio was computed at a number of time points, with the hazard ratio at time t equal to $1.08*0.96^{\ln(t)}$ (Table 3). The effect of pre-operative PCV decreases until day 7 post-operatively then the effect disappears.

Time (days)	Ln(t)	Hazard Ratio
1	0	1.08
3	1.10	1.03
5	1.61	1.01
7	1.95	0.99

Table 3: *Calculation of the hazard ratio in the presence of a PCV*time interaction term at different time points, with hazard ratio at time t equal to* $1.08*0.96^{\ln(t)}$.

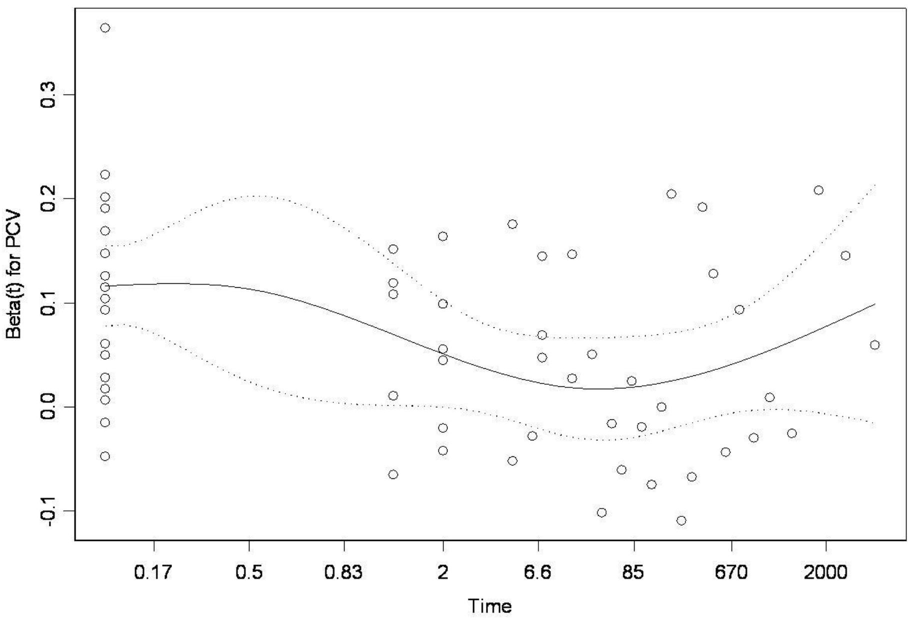


Figure 3: *Schoenfield residual plots for Model one: PCV. This graph reveals a lack of proportionality over survival time, demonstrating that the effect of pre-operative PCV alters with time (p<0.007).*

To illustrate these results, a horse with a LCV that has a PCV on admission of 55% has a 4.7 times greater risk of mortality, intra or post-operatively, than a horse with a pre-operative PCV of 35%.

The results of univariable relationships of potential explanatory pre, intra and post-operative variables with survival time as the outcome measure, when only those horses that recovered successfully from anaesthesia were included (group two) are shown in the appendix to Chapter 2. Categorical variables that were significantly associated with increased risk of post-operative death were colour of large colon serosa (normal/abnormal), colour of mucosa at the enterotomy site (normal/abnormal), haemorrhage at the enterotomy site (present/absent), colic during post-operative hospitalisation (no/yes) and repeat laparotomy (no/yes). Derangements in serosal and mucosal colour and the presence of haemorrhage at the enterotomy site were defined by the surgeon at the time of exploratory laparotomy and recorded in the horse's surgical report. Continuous variables significantly associated with increased risk of post-operative death were pre-operative heart rate and pre-operative PCV, heart rate at 24 and 48 hours post-operatively, PCV at 24 and 48 hours post-operatively and total protein at 24 and 48 hours post-operatively. The final Cox proportional hazards model included serosal colour intra-operatively (normal/abnormal), heart rate at 48 hours post-surgery and colic during post-operative hospitalisation (no/yes), which were all positively associated with risk of post-operative death (Table 4).

Variable	**Coefficient**	**Standard error**	**Hazard ratio**	**95% Confidence Intervals**	**p-value**
Heart rate at 48 hours post-operatively (beats/minute)	0.04	0.01	1.04	1.02-1.06	0.0005
Serosal colour (normal/abnormal)	1.28	0.43	3.61	1.55-8.44	0.003
Colic during post-operative hospitalisation	0.97	0.50	2.63	1.00-6.95	0.05

Table 4: *Final, multivariable Cox proportional hazards model (Model two) for post-operative death in 89 horses with LCV recovering from general anaesthesia.*

Examination of the functional form of the relationship of heart rate at 48 hours post-operatively demonstrated a linearly increasing likelihood of mortality, with no evidence of non-linearity (Fig. 4). In model two graphical and statistical evaluation of Schoenfeld residuals for all variables, and graphical assessment of log cumulative hazard plots for categorical variables, confirmed the assumption of proportional hazards to be valid (see appendix to Chapter 2).

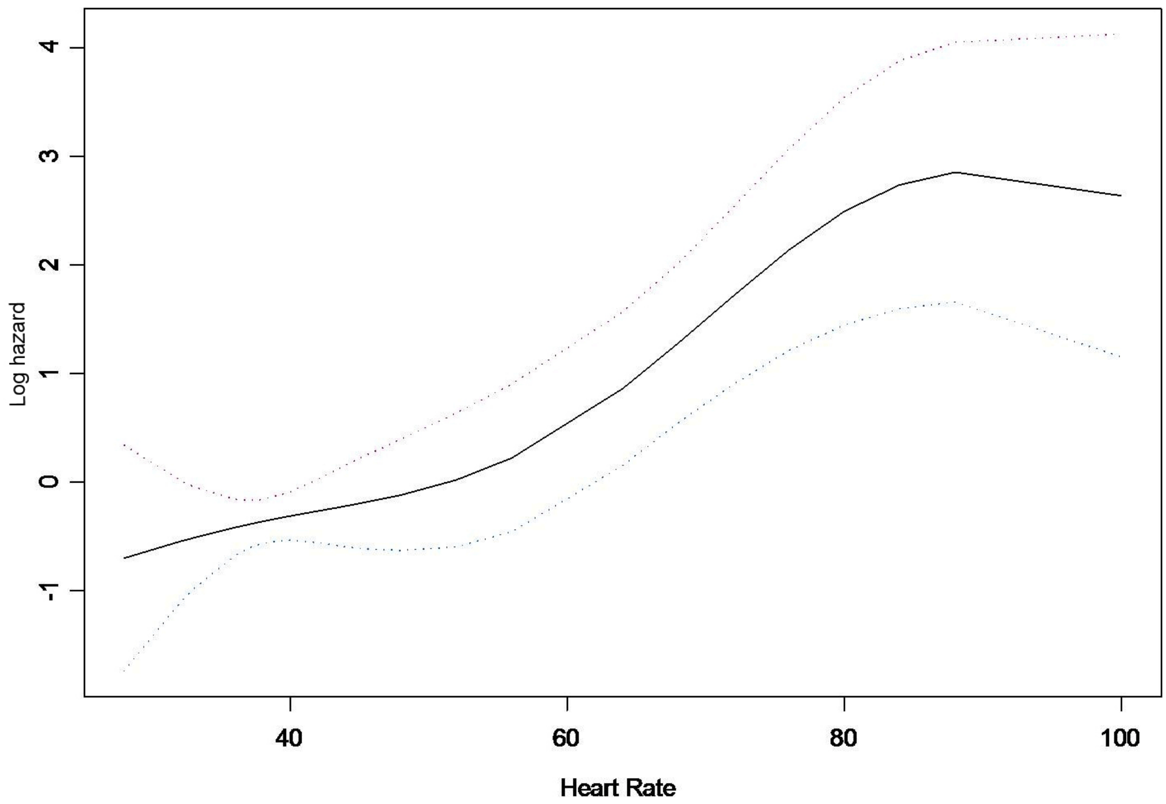


Figure 4: *Graph illustrating the shape of the relationship between heart rate at 48 hours post-operatively and the risk of mortality in 89 horses with LCV that survived anaesthesia. Spline fit (solid line), with 95% confidence intervals (dotted lines) from univariable penalised Cox proportional hazards regression model are illustrated.*

To illustrate the results for model two, a horse with a LCV that has a heart rate of 60 beats per minute at 48 hours post-operatively has a 2.2 times greater risk of post-operative mortality than a horse with a heart rate of 40 beats per minute at 48 hours post-operatively.

For both models removal of influential individual observations with large delta-betas had little effect on coefficients, showing the models were stable; therefore all observations were retained within the models.

Discussion

This study provides information on survival following surgery for a specific type of colic (strangulating LCV) and identifies risk factors for post-operative survival. The results of the study may assist clinicians with decision-making in the management of horses with strangulating LCV and clinicians can utilise this information to provide owners of these horses with evidence-based information regarding their survival following surgery.

Survival to hospital discharge following surgery to correct LCV has been previously reported to lie between 36-74% [9, 10, 11]. This wide range can partially be attributed to differences between hospital populations, the lack of consistency in the definition of a strangulating LCV and the fact that some studies excluded horses that did not survive anaesthesia. Variation in the number of horses euthanised without an opportunity for exploratory laparotomy, due to a poor prognosis, will also influence survival. This study included only horses with a volvulus of greater than or equal to 360 degrees in order to ensure all cases of volvulus were strangulating in nature. LCV was found to be associated with considerable post-operative mortality, with over 20% of horses not recovering from anaesthesia. Of those horses that recovered from anaesthesia, less than 50% were alive one year post-operatively and only a third of horses were alive two years post-operatively.

Two models were created to allow exploration of pre, intra and post-operative parameters. In model one only increased pre-operative packed cell volume (PCV) on admission was significantly associated with increased post-operative mortality. This significant association emphasises the importance of early referral of horses with a suspected LCV, prior to alteration of their PCV. Pre-operative PCV has been shown previously to be significantly associated with long term survival for other colic types [99, 100] and in particular with long term survival following surgery for large intestinal disease [6]. Pre-operative PCV was found to be associated with an increased risk of mortality in the early post-operative period and its influence diminished by day 7. Elevated PCV on admission is likely to reflect the degree of systemic inflammatory response that may develop during hospitalisation, which will affect survival in the immediate post-operative period. Pre-operative PCV was not retained in model two when intra and post-operative variables were included. It is likely that those horses that died or were euthanased on the operating table (and were therefore excluded from model two), had a high PCV on admission, and were excluded from model two.

When pre, intra and post-operative variables were included in a multivariable model, the variables abnormal serosal colour of the large colon intra-operatively, heart rate at 48 hours post-operatively, and colic during post-operative hospitalisation were found to be significantly associated with reduced long term survival. Alteration in serosal colour is indicative of the degree of vascular compromise of the large colon. It may correlate with the degree of the systemic inflammatory response, which occurs as a result of the loss of mucosal barrier function, and the subsequent trans-mural and trans-vascular migration of microbes and pathogen associated molecular patterns, including LPS (endotoxin), into the peritoneal cavity and circulation [68, 69]. Alternative intra-operative methods of estimating prognosis in cases of LCV, including pulse or surface oximetry, fluorometric evaluation [102, 103] and colonic luminal pressure [101] are often unreliable and may not be readily available. Histopathologic evaluation of an intra-operative biopsy, collected from the pelvic flexure, examining the crypts and interstitial crypt space has been shown to be a highly sensitive and specific predictor of post-operative survival in cases of LCV [104, 105]. However, intra-operative evaluation of a biopsy is often impractical and a more recent study found histopathological evaluation of pelvic flexure biopsies did not accurately predict survival [106].

Post-operative persistent tachycardia has been suggested to indicate a poor prognosis for survival in horses with LCV [75]; however, its effect has never previously been quantified. Post-operative tachycardia is related to the degree of systemic inflammatory response present post-operatively [39], which is likely to determine survival in cases of strangulating LCV. Colic during post-operative hospitalisation may be due to localised sites of ischaemia, adhesion formation or stenosis [7]. Physiological derangements of the large intestine may predispose certain individuals to recurrent LCV or large colon displacement [43]. The risk of post-operative colic is significantly increased in horses with LCV [7] and in this study nearly 20% of this population died as a result of colic following discharge from the hospital. Colic during hospitalisation may contribute to a decision for euthanasia during hospitalisation or in other cases be a marker for colic post discharge from the hospital. Further investigation into the incidence of post-operative colic in horses with LCV is warranted.

Variables with over 33% of missing values were excluded from the initial model building procedure. Whilst much of the data included in this study was collected prospectively some data was missing due to the severity of pain on admission and the obvious requirement for immediate exploratory laparotomy. Adequate and complete follow-up is a prerequisite for the conduct of any survival study [139]. The 30-month period during the study where follow-up questionnaires were suspended was due to an alteration in funding, and may have reduced the accuracy of survival time and increased the number of censored individuals. In addition, as in numerous other veterinary studies evaluating survival time, the censorship of horses that were lost to follow-up, and the lack of differentiation between death and euthanasia, may have resulted in erroneous inferences from the data [140, 141]. Horses that were euthanased on the operating table were not excluded from the study population; it was deemed their inclusion would provide clinicians and owners with more accurate information on the prognosis of horses presenting with a strangulating LCV. However, the inclusion of horses that were euthanased on the operating table allows a surgeon's judgment and the owner's financial situation to have a potential influence on survival time. In an attempt to assess the effect of financial constraints on survival, insurance status was assessed within the models, and was found not to be significant (see Appendix to Chapter 2).

Extrapolation of these findings to other populations must be done with caution; over 40% of this population of LCV cases were geldings and the median duration of colic signs prior to admission was 10 hours. The study only included two horses that underwent a large colon resection and anastomosis (LCRA); in our hospital population LCRA has previously been associated with poor survival in horses with large intestinal disease [6]. However, Ellis *et al.* [9] demonstrated favourable survival in horses with strangulating LCV that underwent LCRA, in a population comprising 88% mares with a mean duration of colic signs prior to admission of 4 hours. Whether LCRA improves the prognosis of horses with strangulating LCV requires further investigation [11, 129].

In conclusion this study has demonstrated that LCV of ≥360 degrees is associated with considerable post-operative mortality. Of the horses that recovered from anaesthesia, less than 50% were alive one year post-operatively, and only a third of horses were alive two years post-operatively. Increased pre-operative PCV was significantly associated with increased post-operative mortality, illustrating importance of early referral, prior to alteration in PCV. Alteration in serosal colour intra-operatively, increased heart rate at 48 hours post-operatively, and colic during post-operative hospitalisation were also significantly associated with increased post-operative mortality. The study provides evidence based information on the survival of horses with LCV and identifies parameters that may assist the clinician when determining the prognosis for survival in individual horses.

APPENDIX TO CHAPTER 2

Table 1: *Univariable associations of pre-operative categorical variables (Cox proportional hazards model) with post-operative death in 116 horses with a large colon volvulus ≥360 degrees undergoing exploratory laparotomy.*

Variable		Number (%)	Coefficient	Hazard Ratio	95% Confidence Intervals	P-value	% Missing cases
Breed	TB / TB x and WB / WB x	63 (54)	Ref.				
			-0.395	0.67	0.29-1.59	0.3	10
	Cob / Cob x	13 (11)	0.459	1.58	0.88-2.84		
	Draft / Draft x	19 (16)	0.064	1.07	0.45-2.52		
	Pony / Arab / Arab x	9 (8)					
Gender	Female	61 (53)	Ref.				
	Male	55 (47)	0.223	1.25	0.80-1.96	0.3	0
Crib-biter / windsucking	No	75 (65)	Ref.				
	Yes	11 (10)	-0.241	0.79	0.34-1.84	0.6	26
Previous colic surgery	No	104 (90)	Ref.				
	Yes	3 (3)	-0.383	0.68	0.17-2.79	0.6	8
Insured for vets fees	No	47 (41)	Ref.				
	Yes	63 (54)	0.264	1.30	0.82-2.08	0.3	5
Degree of pain	Not severe	70 (60)	Ref.				
	Severe	44 (38)	0.568	1.76	1.12-2.79	0.02	2

Table 2: *Univariable associations of pre-operative continuous variables (Cox proportional hazards model) with post-operative death in 116 horses with a large colon volvulus ≥360 degrees undergoing exploratory laparotomy.*

Variable	**Unit of measurement**	**Mean / median**	**Range**	**Coefficient**	**Standard Error**	**Hazard Ratio**	**95% Confidence Intervals**	**P-value**	**% Missing cases**
Age	years	11	1-28	0.030	0.023	1.03	0.98-1.08	0.2	0
Weight	kg	583	350-750	0.001	0.001	1.00	1.00-1.00	0.3	11
Duration of colic	hours	10	3-96	-0.004	0.008	1.01	1.00-1.01	0.1	13
Heart Rate	beats/minute	60	32-120	0.024	0.006	1.02	1.01-1.04	<0.001	11
Packed call volume	%	45	27-78	0.061	0.012	1.06	1.04-1.09	<0.001	8
Total Protein	g/litre	67	25-100	0.013	0.016	1.01	0.98-1.05	0.4	13
Lactate	mmol/litre	3.45	0.1-13.2	0.086	0.065	1.09	0.96-1.24	0.2	69

Table 3: *Univariable associations of pre, intra and post-operative categorical variables (Cox proportional hazards model) with post-operative death in 89 horses surviving anaesthesia following surgery for large colon volvulus ≥360 degrees.*

Variable		Number (%)	Coefficient	Hazard Ratio	95% Confidence Intervals	P-value	% Missing cases
Breed	TB / TB x and WB / WB x	48 (54)	Ref.			0.2	0
	Cob / Cob x	11 (12)	-0.381	0.68	0.237-1.967		
	Draft / Draft x	14 (16)	0.668	1.95	0.949-4.010		
	Pony/ Arab /Arab x	7 (8)	0.139	1.15	0.399-3.317		
Gender	Female	48 (54)	Ref.				
	Male	41 (46)	-0.252	0.78	0.444-1.361	0.4	0
Crib-biter / windsucking behaviour	No	62 (70)	Ref.				
	Yes	8 (9)	-0.642	0.53	0.162-1.712	0.3	21
Previous colic surgery	No	80 (90)	Ref.				
	Yes	3 (3)	-0.123	0.89	0.214-3.663	0.9	7
Insured for vets fees	No	39 (43)	Ref.				
	Yes	45 (51)	0.152	1.16	0.659-2.056	0.6	6
Degree of pain	Not severe	59 (66)	Ref.				
	Severe	28 (32)	0.435	1.55	0.863-2.768	0.1	2
Degree of large colon oedema	Absent	7 (8)	Ref.				
	Present	73 (82)	1.028	2.80	0.676-11.557	0.2	10
Colour of serosa	Normal	26 (29)	Ref.				
	Abnormal	37 (42)	1.097	3.00	1.48-6.08	0.002	29

Colour of mucosa	Normal	22 (25)	Ref.				
	Abnormal	21 (24)	0.900	2.46	1.18-5.12	0.02	44
Haemorrhage at enterotomy site	No	2 (2)	Ref.				
	Yes	51 (57)	-3.932	0.020	0.002-0.22	0.001	40
Resection	No	87 (98)	Ref.				
	Yes	2 (2)	-0.249	0.78	0.11-5.67	0.8	0
Enterotomy	No	4 (5)	Ref.				
	Yes	83 (93)	1.140	3.13	0.43-22.70	0.3	2
Pyrexia post-operatively	No	55 (62)	Ref.				
	Yes	20 (23)	-0.051	0.95	0.48-1.89	0.9	16
Plasma	No	63 (71)	Ref.				
	Yes	20 (23)	1.001	2.72	1.45-5.12	0.002	7
Polymixin B	No	68 (76)	Ref.				
	Yes	15 (17)	1.058	1.06	0.47-2.40	0.9	7
Lidocaine	No	75 (84)	Ref.				
	Yes	3 (3)	0.644	1.90	0.46-7.94	0.4	12
Incisional infection	No	62 (70)	Ref.				
	Yes	20 (23)	0.115	1.12	0.56-2.23	0.7	8
Laminitis	No	82 (92)	Ref.				
	Yes	1 (1)	1.867	6.47	0.84-49.74	0.07	7

Thrombophlebitis	No	74 (83)	Ref.				
	Yes	9 (10)	0.655	1.92	0.81-4.58	0.1	7
Diarrhoea	No	73 (82)	Ref.				
	Yes	10 (11)	0.702	2.02	0.93-4.36	0.07	7
Colic during hospitalisation	No	67 (75)	Ref.				
	Yes	19 (21)	1.444	4.24	2.26-7.93	<0.001	3
Repeat laparotomy	No	77 (87)	Ref.				
	Yes	6 (7)	0.963	2.62	1.02-6.72	0.05	7

Table 4: *Univariable associations of pre, intra and post-operative continuous variables (Cox proportional hazards model) with post-operative death, in 89 horses surviving anaesthesia following surgery for large colon volvulus ≥360 degrees.*

Variable	Unit of measurement	Mean / median	Range	Coefficient	Standard Error	HazardRatio	95% Confidence Intervals	P-value	% Missing cases
Age	years	10	1-28	0.022	0.029	1.02	0.96-1.08	0.5	0
Weight	kg	575	350-450	0.001	0.002	1.00	1.00-1.01	0.6	12.4
Duration of colic	hours	11	3-96	0.003	0.008	1.00	1.00-1.02	0.7	14.6
Heart Rate	beats/minute	56	32-108	0.019	0.009	1.02	1.00-1.04	0.03	9
Packed call volume	%	7.9	27-69	0.043	0.018	1.04	1.01-1.08	0.02	7.9
Total Protein	g/litre	66	25-89	0.013	0.021	1.01	0.97-1.06	0.6	11.2
Lactate	mmol/litre	3.0	0.1-10.3	0.028	0.097	1.03	0.85-1.24	0.8	71.9
Mean Arterial Pressure	mmHg	60	30-120	-0.005	0.008	1.00	0.98-1.01	0.5	4.5
Duration of surgery	minutes	90	45-210	0.006	0.004	1.01	1.00-1.01	0.1	0
Heart rate at 24 hours	beats/minute	44	32-104	0.033	0.008	1.03	1.02-1.05	<0.001	13.5
Heart rate at 48 hours	beats/minute	40	28-100	0.051	0.010	1.05	1.03-1.07	<0.001	18

Packed cell volume at 24 hours	%	35	24-61	0.067	0.023	1.07	1.02-1.12	0.003	24.7
Packed cell volume at 48 hours	%	35	26-61	0.131	0.029	1.14	1.08-1.21	<0.001	43.8
Total protein at 24 hours	g/litre	57	36-70	-0.059	0.022	0.94	0.90-0.99	0.009	24.7
Total protein at 48 hours	g/litre	60	44-70	-0.060	0.027	0.94	0.89-0.99	0.03	43.8

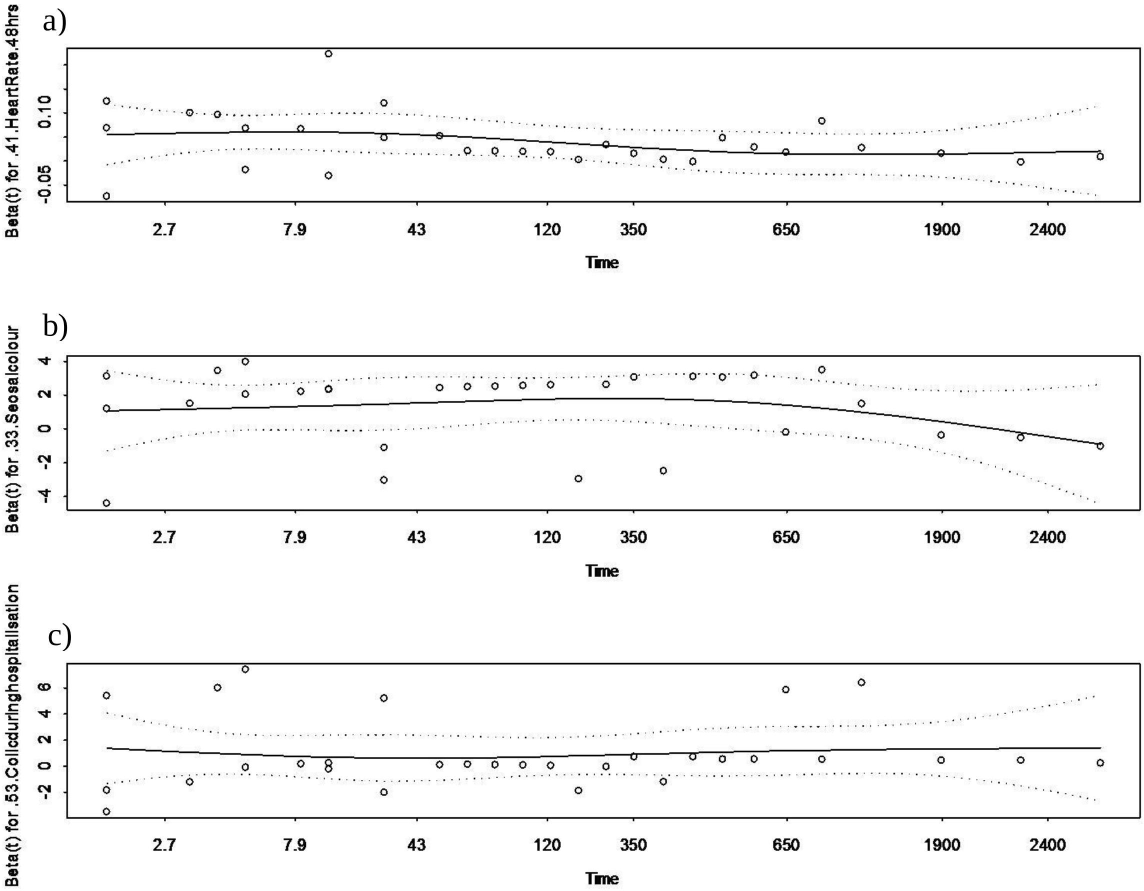
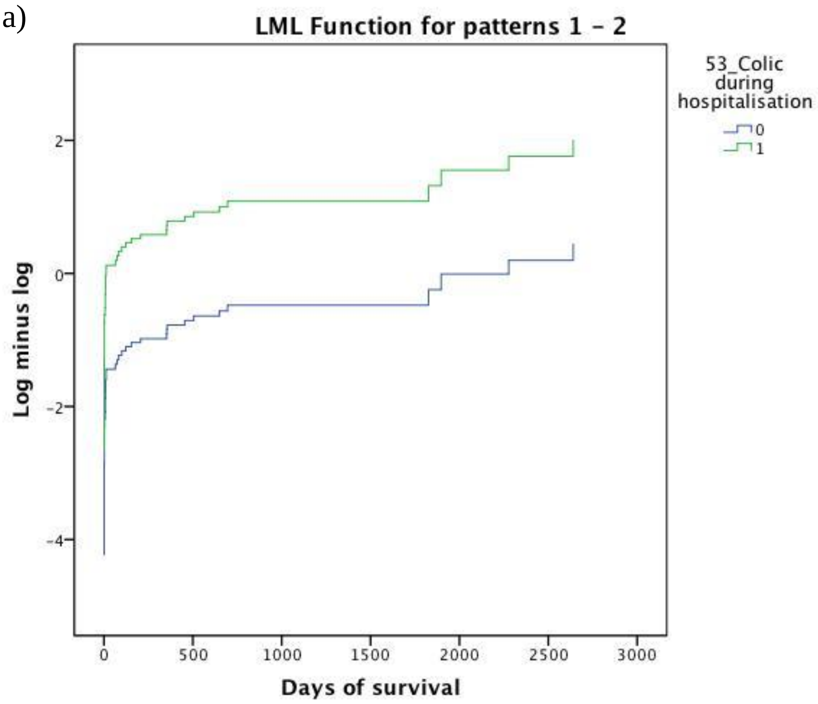


Figure 1: *Schoenfield residual plots for Model two: a) Heart rate at 48hours post-operatively, b) Serosal colour and c) Colic during post-operative hospitalisation. These graphs demonstrate proportionality throughout survival time, with all plots having no significant pattern over time.*

a)



b)

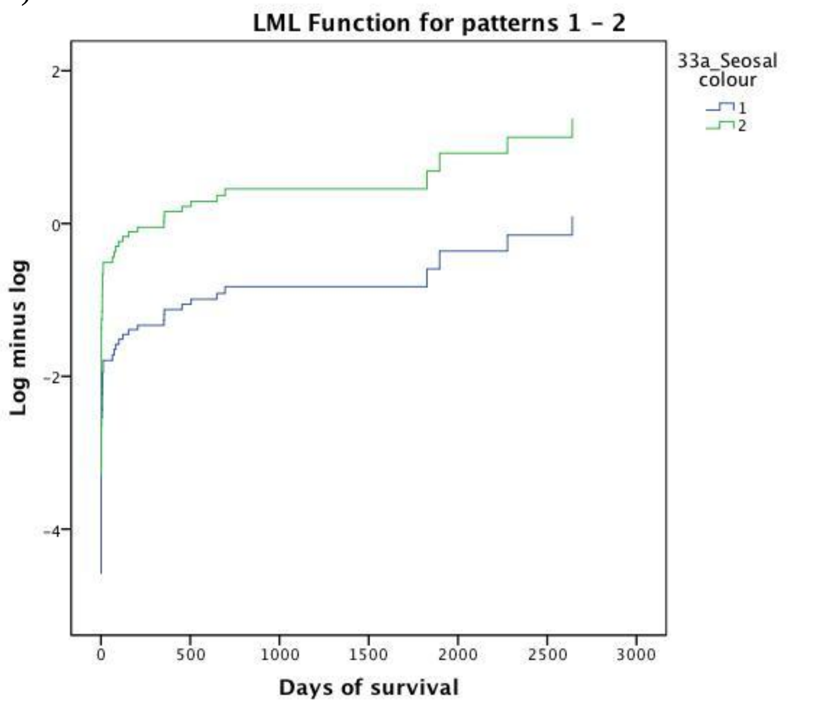


Figure 2a and Figure 2b: *Log minus log cumulative hazard plots for categorical variables in the final model Cox proportional hazards regression model (Model two) for survival in 89 horses surviving anaesthesia following surgery with LCV. These graphs demonstrate proportionality throughout survival time.*

CHAPTER 3

A case-control study to identify risk factors for large colon volvulus

Work presented in this chapter has been accepted for publication during the writing of this thesis (see General Appendix):

Suthers J.M., Pinchbeck G.L., Proudman C.J., Archer D.C. (2013). Risk factors for large colon volvulus in the UK. *Equine Veterinary Journal,* (Article in press).

Abstract

Risk factors for large colon volvulus (LCV) in the horse have not been previously reported. Knowledge of these risk factors may allow the introduction of measures that could be taken to minimise the incidence of LCV. The objectives of this study were to investigate horse and management-level risk factors for LCV in the horse. A prospective, multi-centre, un-matched case-control study was conducted over a 24-month period in the United Kingdom. Data on 69 cases and 204 control horses from four veterinary hospitals were obtained via telephone questionnaires. Multivariable logistic regression was used to identify associations between horse and management-level variables and the likelihood of LCV.

Increasing height, multiple colic episodes in the previous 12 months and mares, with a greater odds ratio in mares that had previously foaled, were associated with increased risk of LCV. A history of receiving medication, (excluding anthelmintic treatment), in the previous 7 days, and quidding behavior were also associated with increased risk of LCV. Management-level variables in the model associated with greater risk of LCV were an increase in the hours of stabling in the previous 14 days, an increasing number of horses on the premises, and 3 or more people involved in the horse's care. Variables related to nutrition within the model associated with increased risk of LCV were being fed hay, being fed sugar-beet, a change in pasture in the previous 28 days, and an alteration in the amount of forage fed in the previous 7 days.

This study has identified factors that may assist in the recognition of horses with increased risk of LCV, and factors that might be altered to minimise the incidence of LCV. Clinicians can utilise this information to identify horses at greater risk of LCV and to provide evidence-based advice to their owners.

Introduction

A successful outcome in cases with strangulating LCV depends on rapid referral and surgical intervention [24]. Despite this surgical correction of LCV is associated with considerable postoperative mortality and morbidity [6, 7] , making identification of susceptible individuals and prevention of LCV very desirable.

Multiple epidemiological studies have identified risk factors associated with colic in general [12, 16, 17, 18, 111], and risk factors associated with specific types of colic. Specific types of colic that have been investigated in epidemiological studies include strangulation of small intestine by pedunculated lipomas [113, 114], ileal impactions [21, 115], simple colonic obstruction/ distension colic (SCOD) [20], impaction colic [116, 117] and epiploic foramen entrapment [13, 14]. Factors identified include horse-level factors, for example height or breed, and modifiable management-level factors, such as exercise regime, feeding, turnout, dental care and anthelmintic treatment. This information has been used to identify high-risk individuals, assist in the diagnosis of specific types of colic and to provide information to owners of horses on strategies to reduce the incidence of colic.

Identification of risk factors for LCV may aid in the understanding of disease causality and has the potential to allow implementation of disease prevention strategies to reduce the incidence of LCV. There are no published reports investigating risk factors specific for LCV. Anecdotal evidence suggests the incidence of large colon displacement or volvulus is increased in Warmblood breeds [121] and that mares in the immediate post-partum period are at increased risk of suffering from LCV [24, 121]. Risk factors for SCOD include a recent change in exercise programme, transport in the preceding 24 hours and crib-biting/windsucking behaviour [20], and one could hypothesise that these may also be risk factors for LCV, as in some cases of LCV the volvulus is proceeded by displacement or impaction of the large colon [24, 25]. The incidence of LCV has been shown to follow a seasonal pattern, with peaks occurring in the spring and autumn [130]. This pattern coincides with periods of management change but its identification does not elucidate specific determinants of LCV [130].

The aim of this study was to identify horse and management-level risk factors for LCV in the UK. It was hypothesised that the post-partum period in broodmares, dietary change, alteration in exercise regime and crib-biting/windsucking behaviour would be associated with increased risk of LCV.

Materials and methods

Study design

A prospective, multi-centre, unmatched, case-control study was conducted to identify associations between horse and management variables and LCV. Four equine hospitals in the United Kingdom were selected on the basis of their caseload, level of surgical expertise and willingness to participate in the study. The hospitals selected for inclusion were Bell Equine Hospital, Donnington Grove Equine Hospital, the University of Liverpool's Philip Leverhulme Equine Hospital and Rossdales Equine Hospital, all of which have specialist surgical facilities and surgical teams headed by one or more Diplomates of the European College of Veterinary Surgeons. The predominant type of horse admitted to the four hospitals varied, with Thoroughbred racehorses representing the majority of horses admitted to Donnington Grove Equine Hospital and Rossdales Equine Hospital, and pleasure horses making up the majority seen at Bell Equine Hospital and the Philip Leverhulme Equine Hospital.

Prior to data collection, the study was approved by the University of Liverpool's ethics committee. Sample size estimation was performed using WinEpiscope 2.0 (www.clive.ed.ac.uk/winepiscope). This indicated that 60 cases and 180 unmatched controls would have 80% power to detect odds ratios of 2.5 or greater, with 95% confidence, assuming 20% exposure in controls. Data from cases and controls were collected over a 24-month period between 1st February 2010 and 1st February 2012. To ensure the cases and controls were unmatched on time, 10 controls were recruited during each month of the study.

Case and control definition and selection

Cases of LCV, greater than or equal to 270 degrees, diagnosed on exploratory laparotomy or at post-mortem examination at the collaborating hospitals, were recruited onto the study. In Chapter 2, where the case definition was retrospective, only horses with a volvulus of greater than or equal to 360 degrees were included to ensure all cases of volvulus were strangulating in nature. However, in this study the inclusion of horses with a strangulating volvulus of greater than or equal to 270 degrees was deemed suitable, since the study was conducted in a prospective manner. Case identification was made by the surgeon, based on the degree of colon wall and mesocolon oedema, the colour of the serosa, and by palpation of the position of the large colon. The first author was notified of a case and if the owner was willing to participate, they were then contacted by telephone to complete a questionnaire (see general appendix). Questionnaires were conducted as soon as possible after exploratory laparotomy, according to clients' and collaborating hospitals' requests.

Control horses were chosen by randomly selecting a client from a list of all clients seen at the collaborating hospitals in the previous year. Due to variation in caseload between the collaborating hospitals, (and therefore variation in size of client list), the number of clients selected as controls from each hospital, was proportional to the caseload of that hospital. Controls were randomly selected at a steady rate throughout the study period to prevent matching on time. To maximise compliance clients were initially sent details of the study by post, and were then contacted by telephone. A control ‘horse’ (horse or pony) was randomly selected from the horses in the client’s care. To avoid selection bias, horses that would not undergo surgical treatment of colic, if it were deemed necessary, were excluded from the study population. In this instance, another horse in the client’s care was randomly selected, or if no suitable horses were available, a new control client was chosen [20].

Data collection

Data were collected using a telephone questionnaire format, which was conducted by one of two individuals, either the author of this thesis or a member of clerical staff, Louisa Platt, each of whom carried out approximately 50% of the questionnaires. The questionnaire was designed utilising information from previous epidemiological studies investigating colic, and hypothesised biologically plausible risk factors [12, 13, 20] (see general appendix). The questionnaire was divided into a number of sections: Signalment and use, medical history, breeding history, premises details, stabling and turnout, nutrition, exercise and transport, behaviour and preventive healthcare. The questionnaire was carefully constructed and piloted to ensure that it was concise and straightforward to complete, in order to maximise compliance and to facilitate the collection of valid, quality data. Following piloting of the questionnaire the terminology utilised in a number of the questions was amended. In addition, two of the questions were re-phrased so that the wording of the questions was less likely to influence a respondent’s answers.

Data were entered onto a data capture form (Teleform v9, Verity Inc.) and a database was created using a data entry scanner (Fujitsu fi-4120C2). Scanned data were manually verified before the data were committed to the database.

To evaluate survival and post-operative colic in the cases of LCV, owners of horses that were discharged from a hospital were contacted, quarterly for the first year following colic surgery, and bi-annually thereafter. Details regarding the incidence of post-operative colic and, if the horse was no longer alive, the reason for death and date of death, were recorded.

Statistical analysis

All explanatory variables were screened for univariable association with LCV. Categorical variables with small numbers of observations in one or more categories, or where the reference category contained relatively few individuals, were re-coded to create fewer categories, or to create a different reference category. The functional form of the relationships between continuous variables and the outcome (LCV) were explored using generalised additive models (GAM) [142]. Prior to multivariable analysis, all variables were assessed for correlation using Spearman's rank correlation coefficients. Where Spearman's rank correlation coefficient was >0.8, the most statistically significant or biologically plausible variable was selected.

Variables showing some evidence of univariable association with outcome ($p \leq 0.3$) were evaluated in a multivariable logistic regression model, which was constructed using a backwards, stepwise elimination procedure [12]. Variables with >20% of missing values were excluded from the initial model-building procedure. Variables remained in the model if they significantly improved the fit ($p \leq 0.05$), assessed using the likelihood ratio statistic. Four sub-models were initially created: Signalment and previous medical history, stabling and behaviour, nutrition and exercise, transport and preventive healthcare. The variables identified in each of these models were then pooled and used to develop a final effects model [16]. All the variables considered for initial inclusion in any of the four sub models were forced back into the model to ensure no significant or confounding variables had been excluded. Variables with >20% missing values were also re-tested in the model at this stage. Biologically plausible interaction terms for variables remaining in the final model were assessed. The fit of the model was assessed using the Hosmer-Lemeshow goodness of fit test statistic. Scaled changes in the regression coefficient for each observation (delta betas) were used to evaluate potential leverage by individual observations for each variable [12]. The model was re-run excluding observations with large delta-beta values (>0.4 or <-0.4) to evaluate their influence on parameter estimates. The critical probability for all analyses was set at 0.05.

Results

Descriptive statistics and univariable analysis

Over the two-year study period, 69 cases of LCV and 204 controls were recruited onto the study. Only two cases of LCV were not recruited onto the study, one because the owner did not wish to participate, and the other because it was not possible to make contact with the client. Of the 280 clients randomly selected to provide a control horse, 23 could not be contacted (due to a change of telephone number or no answer obtained on repeated occasions), 25 did not have a horse that would undergo surgery if it were deemed necessary, and 28 did not wish to participate in the study. This resulted in completion of the questionnaire in 73% of controls and 97% of cases. The telephone questionnaire took a mean of 18 minutes to complete (range 5 – 57 minutes). Cases were identified throughout the 24-

month period, with the greatest number of cases identified in the month of June.

Of the 69 cases of LCV 56 (81%) recovered from anaesthesia. Of those that survived surgery 46 (82%) were discharged from the hospital. Of the cases that were discharged, 24 (52%) had a colic episode reported following discharge, 3 of which had a second LCV identified on exploratory laparotomy. Of those horses that suffered a colic episode following discharge, 8 (33%) were euthanased as a result.

Univariable analysis identified a large number of variables that were significantly associated with LCV (see appendix to Chapter 3, Tables 1-14). Breed, increasing height, multiple colic episodes within the preceeding 12 months, previous large colon volvulus, a medical problem in the last 28 days, receiving a non-steroidal anti-inflammatory drug (NSAID) in the last 28 days, receiving medication, (other than a routine anthelmintic) in the previous 7 days, and a veterinary visit in the previous 7 days were all associated with increased likelihood of LCV.

The variables broodmare (versus male/mare never foaled/mare foaled previously), in foal, and a previous colic episode in the post-partum period, were all significantly associated with increased likelihood of LCV; however, they were highly correlated (Spearman's rank correlation coefficients >0.8) and so broodmare was selected for inclusion in the model. Due to the small number of control horses that had previously foaled (25/204), it was not possible to investigate the association between time since foaling and LCV. Of the cases of LCV that had foaled previously 72% (21/29) had foaled within the proceeding 90 days.

Other variables significantly associated with increased risk of LCV on univariable analysis included type of premises, three or more individuals being involved with the horse's care, an increasing number of horses on a premises, an increase in stabling in the last 14 days, being bedded on straw, a change in pasture in the last 28 days and weaving behaviour. Increasing amount of time exercised per week was associated with a reduced risk of LCV. Variables related to nutrition that were significantly associated with increased the risk of LCV on univariable analysis were periods of the day without access to forage, being fed hay, change in the amount of forage in the previous 7 days, change in the type of forage fed in the previous 28 days, being fed sugarbeet, and a change in the amount of hard feed fed in the previous month. Examination of GAM plots for continuous variables indicated that a linear fit was appropriate for all continuous variables (Figure 1).

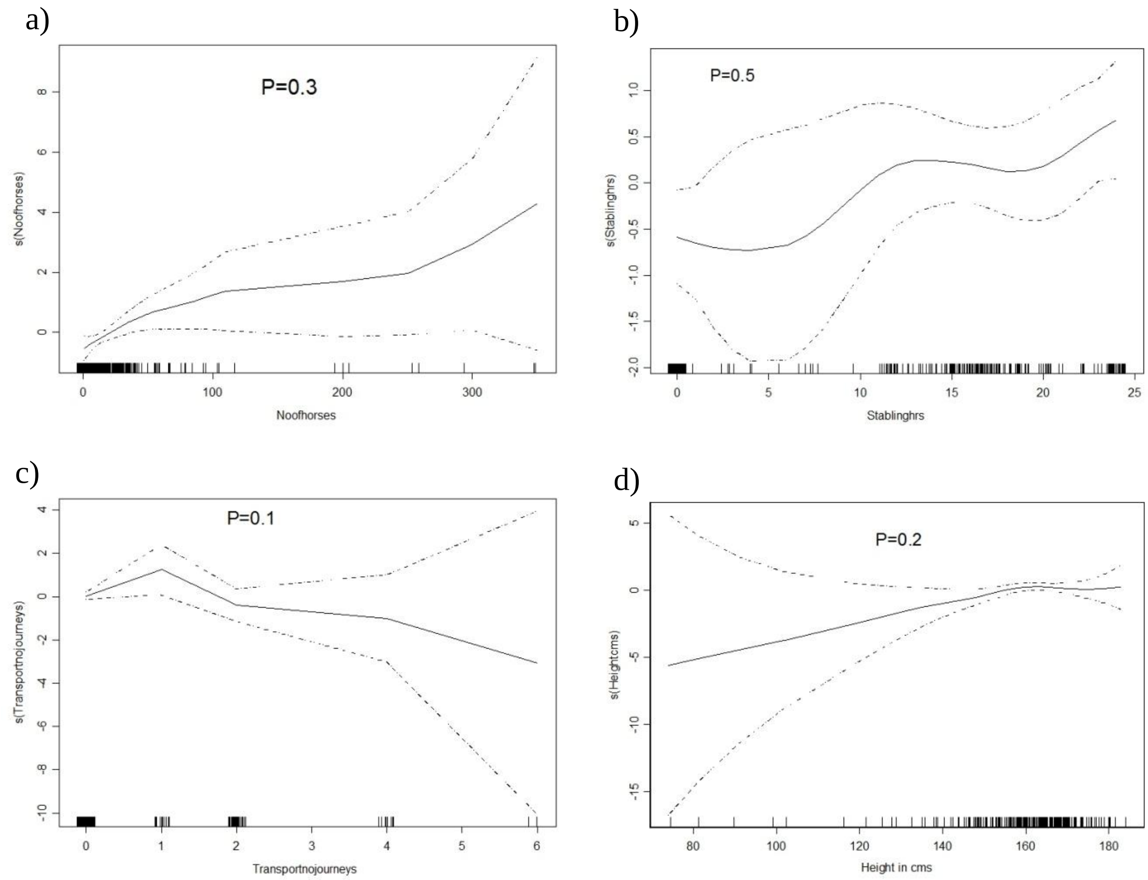


Figure 1: *Use of generalised additive models to demonstrate the functional form of the relationship between the predictor variable and the outcome (log odds of LCV) in a prospective case-control study investigating risk factors for LCV with 69 cases and 204 controls. Predictor variables: a) Number of horses on a premises, b) Hours of stabling in a day, c) Number of times transported in a week, and d) Height in cms. The plots show the fitted curves with 95% confidence intervals (dashed lines) and the rug plots along the x-axis represent the number of data points. The P-value is a chi-square test for non-linearity.*

Multivariable analysis

The final multivariable logistic regression model is shown in Table 1. Increasing height, multiple colic episodes in the previous 12 months, hospital 3 and mares, with a greater odds ratio in mares that had previously foaled, were associated with increased risk of LCV. Management variables in the model associated with an increased likelihood of LCV were an increase in the hours of stabling in the previous 14 days, an increasing number of horses on the premises, and 3 or more people involved in the horse's care. Receiving medication, (excluding anthelmintic treatment), in the previous 7 days, and the horse being noted to quid in the previous 90 days also increased the likelihood of LCV. Variables related to nutrition within the model associated with increased risk of LCV were being fed hay, being fed sugar-beet, a change in pasture in the previous 28 days, and an alteration in the amount of forage fed in the last 7 days.

No significant biologically plausible multiplicative interaction was found between the variables in the final model. The Hosmer-Lemeshow test statistic was 6.76 (P = 0.6, 8 degrees of freedom) indicating no evidence of poor fit. Removal of influential individual observations with large delta-betas had little effect on coefficients, showing the model was stable and all observations were retained.

Variable		Coefficient	Standard Error	Odds Ratio	95% Confidence Interval	Likelihood ratio p-value
Broodmare	Male	Ref.				<0.001
	Mare never foaled	1.52	0.64	4.55	1.30-15.88	
	Mare ≥ 1 foal	2.55	0.72	12.86	3.16-52.27	
Height	cm	0.06	0.03	1.06	1.00-1.12	0.03
>1 colic episode in last 12 months	No	Ref.				0.004
	Yes	2.17	0.81	8.73	1.78-42.74	
Number of carers	< 3	Ref.				<0.001
	≥ 3	2.47	0.59	11.86	3.70-38.02	
Number of horses on premises	per horse	0.01	0.01	1.01	1.00-1.02	0.03
Increase in hours stabled in previous 14 days	No	Ref.				0.04
	Yes	1.70	0.85	5.48	1.03-29.02	
Received medication (excluding anthelmintic) in last 7 days	No	Ref.				0.01
	Yes	1.86	0.74	6.44	1.52-27.36	
Horse noted to quid in last 90 days	No	Ref.				0.005
	Yes	2.05	0.74	7.77	1.82-33.15	
Change in pasture in last 28 days	No	Ref.				
	Yes	1.50	0.58	4.50	1.45-13.92	0.007
Fed hay in last 28 days	No	Ref.				0.004
	Yes	1.54	0.56	4.64	1.54-13.98	
Fed sugar-beet in last 28 days	No	Ref.				0.001
	Yes	1.98	0.63	7.23	2.13-24.62	
Change in amount of forage fed in last 7 days	No	Ref.				
	Yes	2.00	0.882	7.41	1.32-41.71	0.02
Hospital	1	Ref.				<0.001
	2	0.32	0.96	1.38	0.21-8.94	
	3	2.16	0.85	8.70	1.64-46.11	
	4	-1.07	0.88	0.34	0.06-1.94	

Table 1: *Multivariable logistic regression model of risk factors for large colon volvulus in 69 horses from four UK hosptials.*

Discussion

This study is the first epidemiological investigation into horse and management-level risk factors for LCV in the horse. Broodmares, horses that have suffered multiple colic episodes in the previous year, and taller horses are at increased risk of LCV. Other factors identified to significantly increase the risk of LCV include quidding behaviour, feed types and feeding practices, and stabling and turnout. This research provides owners and clinicians with evidence-based information on horses at increased risk of LCV, and on factors that might be altered with the aim of reducing the incidence of LCV.

Mares, and in particular those that have previously foaled, were at increased risk of LCV. Of the cases of LCV that were broodmares, 72% (21/29) had foaled within the last 3 months. This is in agreement with the work of Kaneene *et al.* [17], who found foaling to be significantly associated with increased likelihood of colic in general. A sudden increase in the potential space within the abdomen may explain why mares in the immediate post-partum period are at increased risk. In addition, a variety of management changes occur around the time of foaling, which may increase the risk of LCV in the post-partum period. The correlation between the variables 'foaled previously', 'time since foaling', 'in foal at present', and 'previous colic in the post-partum period' precluded the inclusion of all of these variables in a multivariable model. Further work is required to investigate colic, and in particular LCV, in broodmares in the post-partum period.

A proportionally large potential space within the abdomen may also explain why increasing height was associated with increased risk of LCV. For example, in this study a horse of 173 cm (17 hh) was 8 times more likely to develop LCV compared to a pony of 137 cm (13.2 hh). Horses of greater height were also associated with an increased risk of epiploic foramen entrapment and this was likewise thought to reflect anatomical differences in the relative dimensions within the abdomen [13, 14]. Alternatively the increased risk of LCV with increasing height may be a reflection of differences in the management of ponies and horses; however, this is less likely, since the study took into account other potential confounders such as stabling, turnout, nutrition and exercise. Previously it has been suggested that the incidence of large colon displacement or volvulus is increased in Warmblood breeds [121]. In this study breed was not statistically significant in the final model. However, height did remain in the final model; this may explain why Warmbloods have previously been associated with increased risk of LCV, compared to other, smaller breeds.

This study identified a history of multiple colic episodes within the preceding year to be associated with increased risk of LCV. Several other studies have also shown that horses with a history of colic are at increased risk of suffering further colic episodes [1, 12, 18, 119], lending further strength to this association. Specific to colic associated with the large colon, Hillyer *et al* [20] reported that there is an increased risk of simple colonic obstruction and distention (SCOD) colic in horses with a history of previous colic. These findings may suggest a sub-population of horses exists with underlying gastrointestinal dysfunction affecting the large colon. The density of the interstitial cells of Cajal is reduced in horses with large colon disorders compared to control horses; further studies are required to elucidate mechanisms regulating ICC density and to assess the pathophysiological significance of these findings [43, 143]. Alternatively, it may be that inherent differences between individuals' intestinal microbiota predispose certain horses to recurrent colic and LCV. It has been demonstrated that in horses with simple colonic obstruction and distension (SCOD) colic there is a relative abundance of *Streptococci* and *Lactobaccilli* and a decrease in the proportion of *Fibrobacter spp* [51].

Horses that had received medication, other than a routine anthelmintic, in the previous seven days were at increased risk of LCV. A requirement for medication may be associated with a requirement for management change or physiological derangements, such as pain or inflammation. Furthermore, some medications, such as antimicrobials or non-steroidal anti-inflammatories, may alter colonic microbiota and pH [144, 145, 146, 147], which may explain the increased risk of LCV in those horses that have recently received medication.

A number of alterable management variables were found to increase the risk of LCV. Horses who had an increase in stabling in the previous 14 days were at increased risk. Stabling or reduced time at pasture has been identified as a risk factor for colic in numerous other studies [111, 112, 119], including large colon disorders [20, 117] and large intestinal motility has been shown to be reduced in stabled horses compared to those kept at grass [133]. Increased stabling results in alteration of diet and reduction in activity. Exercise alters gastrointestinal function by decreasing dry matter digestibility of feed, and reducing mean retention time [133, 148, 149]. Our results indicate that where possible pasture turnout should be encouraged, particularly in those horses at increased risk of LCV.

Horses with three or more carers were at increased risk of LCV. This was also identified as a risk factor for large colon impaction in donkeys [117] and may be due to less consistent feeding or management. It has been previously shown that horses whose owners provide their care are at decreased risk of colic [16, 109]; this was attributed to more consistent care. The finding that increased numbers of horses on premises is associated with increased likelihood of LCV may also be attributed to a lack of consistency or individuality in feeding and management.

In this study horses that exhibited quidding behaviour in the previous 90 days were 8 times more likely to develop LCV compared to horses that had not (OR 7.77, 95% CI 1.82 – 33.15). Quidding is often indicative of dental pathology, which has been found to increase the risk of SCOD and recurrent colic in the horse, and of large colon impaction in donkeys [15, 20, 117]. The presence of a dental abnormality on the previous dental examination was not significantly associated with increased risk of LCV; this may be due to the fact that this was owner reported, or because the presence of quidding behaviour is indicative of a relatively severe dental abnormalities. Further research is required to investigate the association of specific dental pathologies with various types of colic and to investigate the cause of these associations. Reduced mastication of feed may lead to ingestion of longer length dietary fibre passing to distal portions of the gastrointestinal tract, increasing the risk of impaction [15, 150], or to an alteration in the composition of ingesta reaching the large colon [15]. Altered mastication of feed can be prevented or reduced by regular, quality dental care. It is important to provide continued education for horse owners, veterinarians and dental technicians regarding the provision of dental care.

Feeding sugarbeet in the previous 28 days increased the risk of LCV. Tinker *et al.* (1997) [18] have previously identified that processed concentrates, such as pellets or sweet feeds increased the risk of colic. Due to missing data, resulting from a lack of accurate information from owners, it was not possible to differentiate between molassed and un-molassed sugar-beet products. This association requires further investigation. An increased risk of large colon volvulus was also associated with a recent change in pasture or change in the amount of forage fed. This may be due to alteration in colonic microbiota and pH following dietary change. Overgrowth of acidophilic *Streptococci* and *Lactobacilli*, will result in an increased concentration of lactate, a reduction in the pH of the luminal environment and a subsequent decrease in the fibrolytic bacterial species [47, 48, 49, 50]. The species that proliferate in a starch rich environment will not only produce excess lactate but also large amounts of CO_2 which will cause distension and pain and potentially result in volvulus of the large intestine [34].

This study identified that those horses that were fed hay, rather than haylage or grass, had an increased the risk of LCV. Management practices or changes, can result in alteration of the luminal environment of the large colon, resulting in dysmotility, impaction and gaseous distension, potentially leading to large colon displacement or volvulus [34, 37, 43, 45, 46]. One could hypothesise that the increased dry matter content of hay, compared to haylage or grass [151], might predispose horses fed hay to impactions and potentially to LCV.

This study used a population of controls that were representative of the population at risk. Control horses were chosen by randomly selecting a client from a list of all clients seen at the collaborating clinics in the previous year. Whilst the number of clients selected as controls from each clinic was proportional to the caseload of that clinic, this did not take into account the differences between the populations of the hospitals, which may explain the increased risk of LCV associated with Hospital 3. In addition, the long-standing reputation of Hospital 3 for referrals for colic surgery may have resulted in horses, with a potentially surgical colic episode that would otherwise be referred to a different hospital, being referred to Hospital 3 resulting in a referral bias. This referral bias may also be a possible explanation for the increased risk of LCV associated with Hospital 3. Alternatively the increased risk associated with Hospital 3 might be because fewer cases were missed at Hospital 3, where the author was based, compared to the number of cases missed at the other hospitals.

Bias during data collection was minimised by following a carefully designed protocol. Recruitment of cases from clinicians experienced in performing exploratory laparotomy avoided potential misclassification bias. Data collection was conducted by one of two investigators, and although case or control status could not be blinded, bias was reduced by developing and piloting a standardised questionnaire. Telephone questionnaires were used to collect data to reduce sampling bias; direct contact improves response rates compared with self-administered questionnaires [152]. The multi-centre nature of the study increased the number of cases collected during the study period and allowed investigation of risk factors for LCV in the UK horse population, rather than in one referral population. Extrapolation of the findings to other horse populations should be done with caution; worldwide differences in genetics, management practices, nutrition and meteorological conditions may influence risk factors for LCV.

In conclusion, this study has identified factors associated with increased risk of LCV. Mares that have previously foaled, horses with a history of multiple colic episodes in the last year, taller horses and horses that have received medication in the previous seven days are all at increased risk of LCV. In addition the study has identified alterable management factors that might be modified to reduce the incidence of LCV, including increasing turnout, not feeding sugarbeet, feeding haylage or grass rather than hay, providing quality dental care to prevent quidding and minimising the number of individuals caring for a horse. An intervention study, potentially in high-risk individuals, such as a population of broodmares, would be required to determine if modification of these factors reduces the incidence of LCV. Clinicians can utilise the information provided by this study to identify horses at risk of LCV, and to provide evidence-based advice to owners of these horses.

APPENDIX TO CHAPTER 3

Table 1: ***Signalment:*** *Univariable associations of categorical variables with large colon volvulus ≥270 degrees.*

Variable		Controls (%)	Cases (%)	Coefficient	Odds Ratio	95% Confidence Intervals	P-value
Hospital	Hospital 1	30 (15)	8 (12)	Ref.			0.1
	Hospital 2	38 (19)	12 (17)	0.169	1.18	0.43 - 3.27	
	Hospital 3	44 (22)	25 (36)	0.756	2.13	0.85 – 5.36	
	Hospital 4	92 (45)	24 (35)	0.459	0.98	0.40 – 2.41	
Gender	Male	126 (62)	23 (33)	Ref.			<0.001
	Female	77 (38)	46 (67)	1.186	3.27	1.84 – 5.82	
Breed	Pony / Other	31 (15)	4 (6)	Ref.			0.002
	TB	39 (19)	29 (42)	1.751	5.76	1.83 – 18.14	
	TB X	49 (24)	10 (15)	0.458	1.58	0.46 – 5.89	
	WB / WB X	45 (22)	10 (15)	0.544	1.72	0.50 – 5.99	
	Shire/ ID / ID X / Clydesdale / Cob	40 (20)	16 (23)	1.131	3.10	0.94 – 10.21	
Body Condition	Normal	139 (68)	37 (57)	Ref.			0.2
	Overweight	45 (22)	18 (28)	0.407	1.50	0.78 – 2.90	
	Lean	20 (10)	10 (15)	0.630	1.88	0.81 – 4.36	
Used for Show-jumping	No	184 (90)	62 (90)	Ref.			0.9
	Yes	20 (10)	7 (10)	0.038	1.04	0.42 – 2.57	
Used for dressage	No	170 (83)	61 (88)	Ref.			0.3
	Yes	34 (17)	8 (12)	-0.422	0.66	0.29 – 1.50	

Used for showing	No	172 (84)	63 (91)	Ref.			0.2
	Yes	32 (16)	6 (9)	-0.670	0.51	0.20 – 1.28	
Used for eventing	No	169 (83)	64 (93)	Ref.			0.05
	Yes	35 (17)	5 (7)	-0.975	0.38	0.14 – 1.01	
Used for hunting	No	191 (94)	67 (97)	Ref.			0.3
	Yes	13 (6)	2 (3)	-0.824	0.44	0.10 – 1.99	
Used for racing	No	195 (96)	68 (99)	Ref.			0.3
	Yes	9 (4)	1 (1)	-1.144	0.32	0.04 – 2.56	
Used for hacking	No	150 (74)	45 (65)	Ref.			0.2
	Yes	54 (27)	24 (35)	0.393	1.48	0.83 – 2.66	
Used in competition	No	126 (62)	50 (73)	Ref.			0.1
	Yes	78 (38)	19 (28)	-0.488	0.61	0.34 – 1.12	
Competes in eventing	No	187 (92)	68 (99)	Ref.			0.08
	Yes	17 (8)	1 (1)	-1.822	0.16	0.01 – 1.24	
Competes in dressage	No	178 (87)	64 (93)	Ref.			0.2
	Yes	26 (13)	5 (7)	-0.626	0.54	0.20 – 1.45	
Competes in Showjumping	No	180 (88)	60 (87)	Ref.			0.8
	Yes	24 (12)	9 (13)	0.118	1.13	0.50 – 2.55	
Competes in Showing	No	186 (91)	67 (97)	Ref.			0.1
	Yes	18 (9)	2 (3)	-1.176	0.31	0.07 – 1.37	

Competes in cross-country / teamchasing	No	199 (98)	68 (99)	Ref.			0.6
	Yes	5 (3)	1 (1)	-0.536	0.59	0.07 – 5.10	
Competes in racing	No	199 (98)	68 (99)	Ref.			0.6
	Yes	5 (3)	1 (1)	-0.536	0.59	0.07 – 5.10	
Competition Level	Not competed	128 (63)	52 (75)	Ref.			0.4
	Local unaffiliated	28 (14)	6 (9)	-0.640	0.53	0.21 – 1.35	
	Low level/ local affiliated	27 (13)	8 (12)	-0.316	0.73	0.31 – 1.71	
	Medium level affiliated	12 (6)	2 (3)	-0.891	0.41	0.09 – 1.90	
	National/ international	9 (3)	1 (1)	-1.296	0.27	0.03 – 2.21	
Insured	No	80 (39)	35 (51)	Ref.			0.1
	Yes	123 (61)	34 (49)	-0.459	0.63	0.37 – 1.10	

Table 2: ***Signalment:*** *Univariable associations of continuous variables with large colon volvulus ≥270 degrees.*

Variable	**Unit of measurement**	**Mean / median**	**Range**	**Coefficient**	**Standard Error**	**Odds Ratio**	**95% Confidence Intervals**	**P-value**
Age	Years	10	1 - 29	0.014	0.027	1.01	0.96-1.07	0.6
In owners possession	Months	48	2 - 1095	-0.002	0.002	1.00	0.99-1.00	0.4
Height	cm	63	74 - 183	0.031	0.014	1.03	1.00 – 1.06	0.03

Table 3: ***Previous medical history:*** *Univariable associations of categorical variables with risk of large colon volvulus ≥270 degrees.*

Variable		Controls (%)	Cases (%)	Coefficient	Odds Ratio	95% Confidence Intervals	P-value
Colic previously	No	148 (73)	36 (52)	Ref.			0.002
	Yes	56 (28)	33 (48)	0.885	2.42	1.38 – 4.26	
Colic episode within last 365 days	No	174 (85)	44 (64)	Ref.			<0.001
	Yes	30 (15)	25 (36)	1.193	3.30	1.76 – 6.16	
Colic episode within last 180 days	No	191 (94)	49 (71)	Ref.			<0.001
	Yes	13 (6)	20 (29)	1.791	6.00	2.79 – 12.89	
Colic episode within last 90 days	No	197 (97)	54 (78)	Ref.			<0.001
	Yes	7 (3)	15 (22)	2.056	7.82	3.03 – 20.14	
Colic episode within last 30 days	No	198 (97)	61 (88)	Ref.			0.009
	Yes	6 (3)	8 (12)	1.465	4.328	1.45 – 12.96	
>1 colic episode in last 365 days	No	199 (98)	57 (83)	Ref.			<0.001
	Yes	5 (3)	12 (17)	2.126	8.38	2.83 – 24.77	
>2 colic episodes in last 365 days	No	201 (99)	65 (94)	Ref.			0.07
	Yes	3 (2)	4 (6)	1.417	4.12	0.90 – 18.91	
Previous abdominal surgery	No	194 (96)	58 (88)	Ref.			0.03
	Yes	9 (4)	8 (12)	1.090	2.97	1.10 – 8.05	
Previous Large colon volvulus	No	202 (99)	63 (93)	Ref.			0.01
	Yes	2 (1)	5 (7)	2.081	8.02	1.52 – 42.33	

Medical problem	No	173 (85)	45 (67)	Ref.			0.002
within last 4 weeks	Yes	31 (15)	22 (33)	1.004	2.73	1.44 – 5.16	
Lameness in last 4	No	184 (91)	62 (93)	Ref.			0.71
weeks	Yes	18 (9)	5 (8)	-0.193	0.82	0.23 – 2.31	
Medication in last 4	No	161 (79)	41 (61)	Ref.		1.31 – 4.31	0.004
weeks	Yes	43 (21)	26 (39)	0.865	2.37		
NSAID in last 4 weeks	No	188 (92)	56 (81)	Ref.			0.01
	Yes	16 (8)	13 (19)	1.003	2.73	1.24 – 6.01	
NSAID in last 7 days	No	193 (96)	61 (88)	Ref.			0.03
	Yes	8 (4)	8 (12)	1.152	3.16	1.14 – 8.79	
Vaccine in last 4 weeks	No	192 (94)	67 (97)	Ref.			0.3
	Yes	12 (6)	2 (3)	-0.739	0.48	0.10 – 2.19	
Antibiotic treatment in	No	199 (98)	64 (93)	Ref.		0.87 – 11.09	0.8
last 4 weeks	Yes	5 (3)	5 (7)	1.134	3.11		
Visited by vet (not for	No	188 (92)	48 (72)	Ref.			<0.001
colic) last 7 days	Yes	16 (8)	19 (28)	1.537	4.65	2.23 – 9.72	
Received medication in	No	191 (94)	54 (78)	Ref.			0.001
last week	Yes	13 (6)	15 (22)	1.406	4.08	1.83 – 9.10	

Days since medical treatment stopped	No medical treatment in last 4 weeks	176 (86)	46 (67)	Ref.			0.007
	Treatment stopped >21 days ago	2 (1)	1 (1)	0.649	1.91	0.17 – 21.56	
	Treatment stopped 15 – 21 days ago	8 (4)	4 (6)	0.649	1.91	0.55 – 6.63	
	Treatment stopped 8 – 14 days ago	5 (3)	3 (4)	0.831	2.30	0.53 – 9.96	
	Treatment stopped within last 7 days	13 (6)	15 (22)	1.485	4.41	1.96 – 9.93	
Received medical treatment in last 7 days	No	191 (94)	54 (78)	Ref.			0.001
	Yes	13 (6)	15 (22)	1.406	4.08	1.83 – 9.10	

Table 4: ***Previous medical history:*** *Univariable associations of continuous variables with large colon volvulus ≥270 degrees.*

Variable	Unit of measurement	Mean / median	Range	Coefficient	Standard Error	Odds Ratio	95% Confidence Intervals	P-value
Length of medical treatment in last 4 weeks	Days	0	0 - 29	0.040	0.020	1.04	1.00 – 1.08	0.04

Table 5: ***Breeding history:*** *Univariable associations of categorical variables with large colon volvulus ≥270 degrees.*

Variable		**Controls (%)**	**Cases (%)**	**Coefficient**	**Odds Ratio**	**95% Confidence Intervals**	**P-value**
In foal at present	Male	127 (62)	23 (33)	Ref.			<0.001
	Mare not in foal	63 (31)	42 (61)	1.303	3.68	2.04 – 6.65	
	Mare in foal	14 (7)	4 (6)	0.456	1.58	0.48 – 5.22	
Broodmare previously	Male	127 (64)	23 (35)	Ref.			<0.001
	Mare never foaled	48 (24)	14 (21)	0.477	1.61	0.77 – 3.39	
	Mare one or more foal	25 (13)	29 (44)	1.857	6.41	3.20 – 12.84	
Colic ≤180 days post-foaling	Mare (including baron)	75 (37)	36 (53)	Ref.			<0.001
	Mare colic post-foaling	1 (1)	9 (13)	2.931	18.75	2.29 – 153.71	
	Gelding or stallion	127 (63)	23 (54)	-0.975	0.38	0.21 – 0.69	
Colic ≤ 90 days post-foaling	Mare (including baron)	75 (37)	37 (54)	Ref.			<0.001
	Mare colic post-foaling	1 (1)	8 (12)	2.786	16.22	1.96 – 134.54	
	Gelding or stallion	127 (63)	23 (34)	-1.002	0.37	0.20 – 0.67	

Table 6: ***Premises:*** *Univariable associations of categorical variables with large colon volvulus ≥270 degrees.*

Variable		**Controls (%)**	**Cases (%)**	**Coefficient**	**Odds Ratio**	**95% Confidence Intervals**	**P-value**
Type of premises	Stud Farm	16 (8)	23 (33)	Ref.			<0.001
	Private yard or field	102 (50)	20 (29)	-1.992	0.14	0.06 – 0.30	
	Livery yard	65 (32)	22 (32)	-1.446	0.24	0.11 – 0.52	
	Professional yard or riding school	20 (10)	4 (6)	-1.972	0.14	0.04 – 0.49	
Main Carer	Owner	161 (79)	42 (61)	Ref.			0.01
	Owner's relative / friend / loaner	9 (5)	4 (6)	0.533	1.70	0.50 – 5.80	
	Professional groom	34 (17)	23 (33)	0.953	2.59	1.38 – 4.86	
Number of premises in last year	One	158 (78)	46 (68)	Ref.			0.1
	Two or more	46 (23)	22 (32)	0.496	1.64	0.90 – 3.01	
Number of days on most recent premises	0 – 300 days	48 (24)	22 (32)	Ref.			0.4
	301 – 730 days	56 (28)	14 (21)	-0.606	0.55	0.25 – 1.18	
	731 – 2190 days	62 (31)	22 (32)	-0.256	0.77	0.38 – 1.56	
	2191 days	37 (18)	10 (15)	-0.258	0.59	0.25 – 1.40	
Number of people involved with horses care	1 or 2 carers	155 (76)	30 (44)	Ref.			<0.001
	3 or more carers	49 (24)	39 (57)	1.414	4.11	2.32 – 7.30	

Table 7: ***Premises:*** *Univariable associations of continuous variables with large colon volvulus ≥270 degrees.*

Variable	Unit of measurement	Mean / median	Range	Coefficient	Standard Error	Odds Ratio	95% Confidence Intervals	P-value
Number of horses on premises	Horse	13	1 – 350	0.013	0.004	1.01	1.01– 1.02	0.001

Table 8: ***Stabling and turnout:*** *Univariable associations of categorical variables with large colon volvulus ≥270 degrees.*

Variable		Controls (%)	Cases (%)	Coefficient	Odds Ratio	95% Confidence Intervals	P-value
Change in hours stabling in last 28 days	No	168 (84)	43 (63)	Ref.			<0.001
	Yes	31 (16)	25 (37)	1.148	3.15	1.67 – 5.88	
Increase in stabling in last 14 days	No	196 (96)	54 (78)	Ref.			<0.001
	Yes	8 (4)	15 (22)	1.918	6.81	2.74 – 16.90	
Increase in stabling in last 7 days	No	198 (97)	60 (87)	Ref.			0.003
	Yes	6 (3)	9 (13)	1.599	4.95	1.69 – 14.47	
Type of stabling change	No change	173 (85)	45 (65)	Ref.			0.001
	Increased stabling	18 (9)	19 (28)	1.401	4.06	1.97 – 8.37	
	Increased turnout	13 (6)	5 (7)	0.391	1.48	0.50 – 4.36	
Bedding type	Not stabled	63 (31)	10 (15)	Ref.			0.001
	Straw	53 (26)	36 (54)	1.545	4.28	1.94 – 9.43	
	Shavings	66 (33)	18 (27)	0.541	1.72	0.74 – 4.01	
	Other	20 (10)	3 (5)	-0.57	0.95	0.24 – 3.77	
Bedded on straw	No	149 (74)	31 (46)	Ref.			<0.001
	Yes	53 (26)	36 (54)	1.183	3.27	1.84 – 5.793	
Bedded on shavings	No	136 (67)	49 (73)	Ref.			0.4
	Yes	66 (33)	18 (27)	-0.278	0.76	0.41 – 1.40	
Bedded on paper or cardboard	No	198 (98)	66 (99)	Ref.			0.8
	Yes	4 (2)	1 (2)	-0.288	0.75	0.08 – 6.83	

Bedded on wood-fibre	No	187 (93)	66 (99)	Ref.			0.1
	Yes	15 (7)	1 (2)	-1.667	0.19	0.02 – 1.46	
Eats bedding	No	160 (78)	53 (79)	Ref.			0.9
	Yes, on previous bedding	19 (9)	7 (10)	0.106	1.11	0.44 – 2.79	
	Yes, on current bedding	25 (12)	7 (10)	-0.168	0.85	0.35 – 2.07	
Turnout in sand-paddock	No	198 (97)	64 (94)	Ref.			0.3
	Yes	6 (3)	4 (6)	0.724	2.06	0.56 – 7.54	
Turnout in grass-paddock	No	20 (10)	10 (15)	Ref.			0.3
	Yes	184 (90)	58 (85)	-0.461	0.63	0.28 – 1.42	
Pasture condition	No grass, not turned out	21 (10)	8 (12)	Ref.			0.9
	Short grass	109 (53)	32 (49)	-0.261	0.77	0.31 – 1.90	
	Mid-length grass	66 (32)	24 (36)	-0.47	0.96	0.37 – 2.44	
	Long grass	8 (4)	2 (3)	-0.42	0.66	0.11 – 3.78	
Sandy soil	No	154 (80)	45 (78)	Ref.			0.7
	Yes	39 (20)	13 (22)	0.132	1.14	0.56 – 2.32	
Change in pasture in last 28 days	No	156 (78)	39 (61)	Ref.			0.009
	Yes	45 (22)	25 (39)	0.799	2.22	1.22 – 4.06	
Number of pastures in last 28 days	No change in pasture	159 (78)	45 (65)	Ref.			0.02
	In 2 pastures	40 (20)	17 (25)	0.407	1.50	0.78 – 2.90	
	In 3 or more pastures	5 (3)	7 (10)	1.599	4.95	1.50 – 16.33	
Change in quality of pasture	No change	175 (86)	57 (83)	Ref.		0.61 – 15.64	0.4
	Change in quality of pasture	29 (14)	12 (17)	0.239	1.27		

Table 9: ***Stabling and turnout:*** *Univariable associations of continuous variables with large colon volvulus ≥270 degrees.*

Variable	Unit of measurement	Mean / median	Range	Coefficient	Standard Error	Odds Ratio	95% Confidence Intervals	P-value
Hours of stabling	Hours	15	0 -24	0.050	0.017	1.05	1.02 –1.09	0.004

Table 10: ***Exercise and transport:*** *Univariable associations of categorical variables with large colon volvulus ≥270 degrees.*

Variable		Controls (%)	Cases (%)	Coefficient	Odds Ratio	95% Confidence Intervals	P-value
In routine work	No	69 (34)	39 (57)	Ref.			0.001
	Yes	135 (66)	29 (43)	-0.967	0.38	0.22 – 0.67	
Exercise intensity	Not in work	71 (35)	40 (58)	Ref.			0.003
	Light exercise	54 (27)	9 (13)	-1.218	0.30	0.13 – 0.66	
	Moderate exercise	49 (24)	17 (25)	-0.485	0.62	0.31 – 1.21	
	Hard exercise	30 (15)	3 (4)	-1.729	0.18	0.05 – 0.62	
Change in the duration of work in last 28 days	No	163 (80)	52 (75)	Ref.			0.4
	Yes	41 (20)	17 (25)	0.262	1.30	0.68 – 2.48	
Gradual or sudden change in duration of work	No change	163 (80)	52 (75)	Ref.			0.4
	Gradual change	26 (13)	8 (12)	-0.36	0.96	0.41 – 2.26	
	Sudden change	15 (7)	9 (13)	0.632	1.88	0.78 – 4.55	
Type of change in duration of work	No change	163 (80)	52 (75)	Ref.			0.2
	Increased duration	26 (13)	7 (10)	-0.170	0.84	0.35 – 2.06	
	Decreased duration	15 (7)	10 (15)	0.737	2.09	0.89 – 4.93	
Overall change in duration of work in last 28 days	No change	163 (80)	52 (75)	Ref.			0.1
	Sudden decrease	11 (5)	6 (9)	0.536	1.71	0.60 – 4.85	
	Gradual decrease	3 (2)	4 (6)	1.430	4.18	0.91 – 19.29	
	Sudden Increase	4 (2)	3 (4)	0.855	2.35	0.51 – 10.85	
	Gradual increase	23 (11)	4 (6)	-0.607	0.55	0.18 – 1.65	
Decrease in duration of work in last 28 days	No	190 (93)	59 (86)	Ref.			0.06
	Yes	14 (7)	10 (15)	0.83	2.30	0.97 – 5.45	

Change in duration of work in last 14 days	No	186 (91)	61 (88)	Ref.			0.5
	Yes	18 (9)	8 (12)	0.304	1.36	0.56 – 3.27	
Change in duration of work in last 7 days	No	194 (95)	64 (93)	Ref.			0.5
	Yes	67 (5)	5 (7)	0.416	1.52	0.50 – 4.60	
Alteration in intensity of work in last 28 days	No	161 (79)	39 (77)	Ref.			0.7
	Yes	42 (21)	12 (24)	0.165	1.18	0.59 – 2.45	
Sudden or gradual alteration in intensity of work	No	161 (79)	39 (77)	Ref.			0.2
	Gradual change	29 (14)	5 (10)	-0.340	0.71	0.26 – 1.96	
	Sudden change	13 (6)	7 (14)	0.799	2.22	0.83 – 5.94	
Type of change of intensity of work	No change	161 (79)	57 (83)	Ref.			0.8
	Increased intensity	27 (13)	7 (10)	-0.312	0.73	0.30 – 1.77	
	Decreased intensity	15 (7)	5 (7)	-0.060	0.94	0.33 – 2.71	
Overall change in intensity of work in last 28 days	No change	161 (79)	57 (83)	Ref.			0.1
	Sudden decrease	12 (6)	3 (4)	-0.348	0.71	0.19 – 2.59	
	Gradual decrease	3 (2)	2 (3)	0.633	1.88	0.31 – 11.56	
	Sudden increase	2 (1)	4 (6)	1.732	5.65	1.01 – 31.68	
	Gradual increase	25 (12)	3 (4)	-1.082	0.339	0.10 – 1.17	
Exercised within 2 hours of feeding in last 48 hours	No	178 (87)	47 (89)	Ref.			0.9
	Yes	26 (13)	6 (11)	-0.135	0.87	0.34 – 2.25	
Transported in last 7 days	No	158 (78)	54 (79)	Ref.			0.7
	Yes	46 (23)	14 (21)	-0.116	0.89	0.45 – 1.75	
Transported in last 24 hours	No	189 (93)	66 (96)	Ref.			0.4
	Yes	15 (7)	3 (4)	-0.557	0.57	0.16 – 2.05	

Transported in last 48 hours	No	180 (88)	64 (93)	Ref.			0.3
	Yes	24 (12)	5 (7)	-0.535	0.59	0.16 – 2.04	

Table 11: ***Exercise and Transport:*** *Univariable associations of continuous variables with large colon volvulus ≥270 degrees.*

Variable	Unit of measurement	Mean / median	Range	Coefficient	Standard Error	Odds Ratio	95% Confidence Intervals	P-value
Number of hours worked in a week	Hours	2.5	0 – 12	-0.159	0.059	0.85	0.76 – 0.96	0.007
Number of times worked in a week	Per exercise session	3	0 - 7	-0.209	0.058	0.81	0.72 – 0.91	<0.001
Number of journeys in last 7 days	Per journey	0	0 - 6	-0.201	0.153	0.82	0.61 – 1.10	0.2
Total transport over last 7 days	Minutes	0	0 - 480	-0.002	0.003	1.00	0.99 – 1.00	0.5

Table 12: ***Behaviour:*** *Univariable associations of categorical variables with large colon volvulus ≥270 degrees.*

Variable		Controls (%)	Cases (%)	Coefficient	Odds Ratio	95% Confidence Intervals	P-value
Restless when stabled	No	175 (86)	57 (86)	Ref.			1.0
	Yes	28 (14)	9 (14)	-0.013	0.99	0.44 – 2.22	
Distressed when left alone in stable	No	150 (76)	53 (86)	Ref.			0.1
	Yes	48 (24)	9 (15)	-0.634	0.53	0.24 – 1.16	
Behavior prior to feeding	Shows normal amount of interest	142 (77)	49 (78)	Ref.			0.4
	Becomes very agitated	28 (15)	12 (19)	0.217	1.24	0.59 – 2.63	
	Shows little or no interest	14 (8)	2 (3)	-0.882	0.41	0.09 – 1.89	
Speed at which eats concentrates	Eats all feed at normal speed	128 (70)	42 (68)	Ref.			0.5
	Very quickly, bolts feed	17 (9)	9 (15)	0.478	1.61	0.67 – 3.89	
	Eats slowly, picks at feed	38 (21)	11 (18)	-0.125	0.88	0.41 – 1.88	
Speed at which eats forage	Eats all forage but not at once	117 (67)	49 (78)	Ref.			0.1
	Eats all forage very quickly	30 (17)	10 (16)	-0.228	0.80	0.36 – 1.75	
	Doesn't eat all forage	29 (17)	4 (6)	-1.111	0.33	0.11 – 0.99	
Feeding patterns altered when stressed	No, eats as normal	159 (90)	54 (93)	Ref.			0.5
	Yes, will go off feed	17 (10)	4 (7)	-0.367	0.69	0.22 – 2.15	
Able to see other horses from stable	No	10 (5)	1 (2)	Ref.			0.2
	Yes	194 (95)	66 (99)	1.224	3.40	0.43 – 27.08	
Able to groom other horses	No	44 (22)	15 (22)	Ref.			0.9
	Yes	160 (78)	52 (78)	-0.048	0.95	0.49 – 1.85	

Crib-biting or windsucking behaviour	No	190 (93)	59 (87)	Ref.			0.1
	Yes	14 (7)	9 (13)	0.728	2.07	0.85 – 5.03	
Box-walking	No	196 (96)	64 (94)	Ref.			0.5
	Yes	8 (4)	4 (6)	0.426	1.53	0.45 – 5.25	
Weaving	No	193 (95)	59 (87)	Ref.			0.04
	Yes	11 (5)	9 (13)	0.984	2.68	1.06 – 6.77	
Any stereotypic behaviour	No	173 (85)	52 (75)	Ref.			0.03
	Yes	31 (15)	17 (25)	0.601	1.82	0.94 – 3.56	
When stereotypic behaviour exhibited	No vice ever seen	172 (85)	53 (77)	Ref.			0.1
	Only when stabled / left alone	25 (12)	7 (10)	-0.096	0.91	3.72 – 2.22	
	Both when stabled and turned out	6 (3)	9 (13)	1.583	4.87	1.66 – 14.31	
Frequency stereotypic behaviour exhibited	No	173 (85)	53 (77)	Ref.			0.2
	Seen on rare / specific occasions	13 (6)	4 (6)	0.004	1.00	0.31 – 3.21	
	Seen once a week but not every day	7 (3)	3 (4)	0.336	1.40	0.35 – 5.60	
	Seen every day for short periods	8 (4)	4 (6)	0.490	1.63	0.47 – 5.63	
	Seen every day for prolonged periods	3 (2)	5 (7)	1.694	5.44	1.26 – 23.52	

Table13: ***Preventative health care:*** *Univariable associations of categorical variables with large colon volvulus ≥270 degrees.*

Variable		Controls (%)	Cases (%)	Coefficient	Odds Ratio	95% Confidence Intervals	P-value
Approximate date of last anthelmintic treatment known	No	19 (9)	12 (17)	Ref.			0.07
	Yes	185 (91)	57 (83)	-0.718	0.49	0.22 – 1.07	
Most recent anthelmintic treatment known	No	56 (28)	22 (32)	Ref.			0.5
	Yes	148 (73)	47 (68)	-0.213	0.80	0.45 – 1.46	
Most recent anthelmintic used	Moxidectin	36 (24)	8 (17)	Ref.			0.5
	Ivermectin + Praziquantel	44 (30)	13 (28)	0.285	1.33	0.50 – 3.56	
	Fenbendazole	6 (4)	6 (13)	1.504	4.50	1.15 – 17.65	
	Ivermectin / Doramectin	21 (14)	6 (13)	0.251	1.29	0.39 – 4.21	
	Moxidectin + Praziquantel	30 (20)	9(20)	0.300	1.35	0.46 – 3.93	
	Praziquantel	3 (2)	2 (4)	1.099	3.00	0.43 – 21.01	
	Pyrantel	8 (5)	2 (4)	0.118	1.125	0.20 – 6.34	
Praziquantel or double dose pyrantel in last 180 days	No	46 (31)	9 (20)	Ref.			0.2
	Yes	105 (70)	37 (80)	0.59	1.80	0.80 – 4.04	
Ivermectin or moxidectin in previous 365 days	No	12 (8)	6 (13)	Ref.			0.4
	Yes	136 (92)	42 (88)	-0.482	0.62	0.22 – 1.75	
Individual who performed most recent dental examination	Vet	63 (34)	17 (30)	Ref.			0.6
	Equine Dentist	122 (66)	39 (70)	0.169	1.19	0.62 – 2.26	

Dental gag used	No	9 (5)	6 (12)	Ref.			0.1
	Yes	160 (95)	45 (88)	-0.863	0.42	0.14 – 1.23	
Light source used	No	24 (16)	10 (28)	Ref.			0.1
	Yes	128 (84)	26 (72)	-0.718	0.49	0.21 – 1.14	
Dental abnormalities detected	No	157 (84)	40 (76)	Ref.			0.2
	Yes	30 (16)	13 (25)	0.531	1.70	0.81 – 3.56	
Quidding evident	No	187 (94)	54 (86)	Ref.			0.06
	Yes	13 (7)	9 (14)	0.874	2.40	0.97 – 5.91	
Days since last anthelmintic treatment	0 – 30 days	72 (40)	22 (39)	Ref.			0.6
	31 – 55 days	20 (11)	8 (14)	0.269	1.31	0.51 – 3.38	
	56 – 90 days	67 (36)	16 (28)	-0.246	0.78	0.38 – 1.61	
	>90 days	26 (14)	11 (19)	0.325	1.39	0.59 – 3.24	
Days since last dental examination	0 – 90 days	48 (25)	18 (30)	Ref.			0.7
	91 – 180 days	70 (36)	17 (28)	-0.434	0.65	0.30 – 1.38	
	181 – 300 days	31 (16)	10 (16)	-0.151	0.86	0.35 – 2.11	
	>300 days	45 (23)	16 (26)	-0325	0.95	0.43 – 2.08	
Days since last dental examination	0 – 365 days	172 (89)	50 (82)	Ref.			0.1
	>365 days	21 (11)	11 (18)	0.589	1.80	0.81 – 3.99	

Table 14: ***Nutrition:*** *Univariable associations of categorical variables with large colon volvulus ≥270 degrees.*

Variable		**Controls (%)**	**Cases (%)**	**Coefficient**	**Odds Ratio**	**95% Confidence Intervals**	**P-value**
Number of times fed forage per day	Grass only	42 (21)	5 (8)	Ref.			0.1
	Once daily	60 (29)	17 (26)	0.867	2.38	0.82 – 6.95	
	Twice daily	58 (28)	24 (36)	1.246	3.48	1.23 – 9.86	
	Three times daily	18 (9)	9 (14)	1.435	4.20	1.23 – 14.29	
	Ad-lib	26 (13)	11 (17)	1.268	3.55	1.11 – 11.39	
Periods of the day when without forage	No	87 (44)	16 (26)	Ref.			0.01
	Yes	110 (56)	45 (74)	0.800	2.22	1.78 – 4.20	
Fed forage	No	42 (21)	5 (8)	Ref.			0.02
	Yes	162 (79)	61 (92)	1.152	3.16	1.20 – 8.37	
Fed dry hay	No	126 (62)	31 (46)	Ref.			0.02
	Yes	77 (38)	37 (54)	0.67	1.95	1.12 – 3.40	
Fed soaked hay	No	182 (90)	57 (84)	Ref.			0.2
	Yes	21 (10)	11 (16)	0.514	1.67	0.76 – 3.38	
Fed hay	No	106 (52)	20 (29)	Ref.			0.001
	Yes	97 (48)	48 (71)	0.964	2.62	1.45 – 4.73	
Fed haylage	No	129 (64)	48 (71)	Ref.			0.3
	Yes	74 (37)	20 (29)	-0.320	0.73	0.40 – 1.32	
Amount of forage changed in last 28 days	No	167 (82)	47 (69)	Ref.			0.03
	Yes	37 (18)	21 (31)	0.701	2.02	1.08 – 3.77	

Amount of forage changed in last 14 days	No	180 (89)	51 (76)	Ref.			0.01
	Yes	23 (11)	16 (24)	0.898	2.46	1.21 – 4.99	
Amount of forage changed in last 7 days	No	197 (97)	57 (86)	Ref.			0.003
	Yes	6 (3)	9 (14)	1.65	5.18	1.77 – 15.18	
Increase or decrease in amount of forage in the last 28 days	No change	167 (82)	48 (70)	Ref.			0.1
	Increased amount	23 (11)	12 (17)	0.596	1.82	0.84 – 3.91	
	Decreased amount	14 (7)	9 (13)	0.805	2.24	0.91 – 5.48	
Speed of change in forage in last 28 days	No change	168 (82)	49 (71)	Ref.			0.04
	Gradual change	16 (8)	5 (7)	0.069	1.07	0.37 – 3.07	
	Sudden change	20 (10)	15 (22)	0.944	2.57	1.23 – 5.40	
Type of forage change in last 28 days	No change	168 (82)	49 (71)	Ref.			0.1
	Sudden decrease	6 (3)	6 (9)	1.232	3.43	1.06 – 11.11	
	Gradual decrease	8 (4)	2 (3)	-0.154	0.86	0.18 – 4.17	
	Sudden increase	14 (7)	9 (13)	0.790	2.20	0.90 – 5.40	
	Gradual increase	8 (3)	3 (4)	2.51	1.29	0.33 – 5.03	
Altered frequency of feeding forage in last 28 days	No	181 (89)	54 (79)	Ref.			0.04
	Yes	22 (11)	14 (21)	0.758	2.13	1.02 – 4.45	
Altered frequency of feeding forage in last 14 days	No	187 (92)	57 (84)	Ref.			0.05
	Yes	16 (8)	11 (16)	0.813	2.26	0.99 – 5.14	
Altered frequency of feeding forage in last 7 days	No	194 (96)	62 (91)	Ref.			0.2
	Yes	9 (4)	6 (9)	0.735	2.09	0.71 – 6.09	
Increase or decrease in frequency of forage feeding in last 28 days	No change	182 (90)	55 (80)	Ref.			0.1
	Increased frequency	12 (6)	9 (13)	0.909	2.48	0.99 – 6.20	
	Decreased frequency	9 (4)	5 (7)	0.609	1.84	0.59 – 5.71	

Change in type of forage fed in last 28 days	No change	193 (95)	58 (85)	Ref.			0.01
	Change in type of forage	10 (5)	10 (15)	1.202	3.33	1.32 – 8.39	
Change in type of forage fed in last 14 days	No change	197 (97)	60 (88)	Ref.			0.05
	Change in type of forage	6 (13)	8 (12)	0.813	2.26	0.99 – 5.14	
Change in type of forage fed in last 7 days	No change	199 (98)	63 (93)	Ref.			0.2
	Change in type of forage	4 (2)	5 (7)	0.735	2.09	0.71 – 6.09	
Change in the batch of forage fed in last 28 days	No change	156 (79)	49 (79)	Ref.			1.00
	Change in batch of forage	41 (21)	13 (21)	0.009	1.01	0.50 – 2.04	
Number of times fed hard feed per day	Not fed hard feed	29 (14)	7 (11)	Ref.			0.3
	Once or twice daily	158 (78)	50 (76)	0.271	1.31	0.54 – 3.18	
	Three or more times daily	16 (8)	9 (14)	0.846	2.33	0.73 – 7.44	
Fed concentrate feed	No	55 (27)	14 (21)	Ref.			0.3
	Yes	147 (73)	52 (79)	0.329	1.39	0.71 – 2.71	
Frequency of feeding concentrates	Not fed concentrate	54 (27)	18 (27)	Ref.			0.07
	Once daily	35 (17)	4 (6)	-1.070	0.343	0.11 – 1.10	
	Twice daily	101 (50)	37 (54)	0.094	1.10	0.57 – 2.11	
	Three of more times daily	13 (6)	9 (13)	0.731	2.08	0.76 – 5.67	
Fed grain	No	183 (91)	60 (91)	Ref.			0.9
	Yes	19 (9)	6 (9)	-0.038	0.96	0.37 – 2.52	
Frequency fed grain	Not fed grain	184 (91)	62 (91)	Ref.			1.00
	Once daily	6 (3)	2 (3)	-0.011	0.99	0.20 – 5.03	
	Twice daily	10 (5)	4 (6)	0.172	1.88	0.36 – 3.92	
	Three of more times daily	3 (2)	0 (0)	-20.115	0.000		

Fed sugar-beet	No	179 (89)	47 (71)	Ref.			0.001
	Yes	22 (11)	19 (29)	1.191	3.29	1.65 – 6.58	
Frequency fed sugar-beet	Not fed sugar-beet	180 (89)	49 (73)	Ref.			0.008
	Once daily	8 (4)	3 (5)	0.320	1..38	0.35 – 5.39	
	Twice daily	12 (6)	14 (21)	1.46	4.29	1.86 – 9.86	
	Three of more times daily	3 (2)	1 (2)	0.20	1.22	0.13 – 12.03	
Fed short chop fibre	No	65 (32)	31 (47)	Ref.			0.03
	Yes	136 (68)	35 (53)	-0.617	0.54	0.31 – 0.95	
Frequency fed short chop fibre	Not fed short-chop fibre	73 (36)	33 (49)	Ref.			0.06
	Once daily	31 (15)	4 (6)	-1.254	0.29	0.09 – 0.87	
	Twice daily	92 (45)	25 (37)	-0.509	0.60	0.33 – 1.10	
	Three of more times daily	8 (4)	5 (8)	0.324	1.38	0.42 – 4.55	
Change in amount of hard feed in last 28 days	No	178 (89)	46 (70)	Ref.			<0.001
	Yes	23 (11)	20 (30)	1.213	3.37	1.70 – 6.65	
Change in amount of hard feed in last 14 days	No	186 (93)	55 (83)	Ref.			0.03
	Yes	15 (8)	11 (17)	0.908	2.48	1.08 – 5.71	
Change in amount of hard feed in last 7 days	No	193 (96)	64 (97)	Ref.			0.7
	Yes	8 (4)	2 (3)	-0.282	0.75	0.16 – 3.64	
Type of change in amount of hard feed in last 28 days	No change	181 (89)	50 (74)	Ref.			0.06
	Sudden decrease	5 (3)	3 (4)	0.776	2.17	0.50 – 9.40	
	Gradual decrease	5 (3)	4 (6)	1.063	2.89	0.75 – 11.19	
	Sudden increase	5 (3)	5 (7)	1.286	3.62	1.01 – 13.00	
	Gradual increase	8 (4)	6 (9)	0.999	2.72	0.90 – 8.19	

Increase or decrease in amount of hard feed in last 28 days	No change	181 (89)	50 (74)	Ref.			0.1
	Decrease	10 (5)	7 (10)	0.930	2.53	0.92 – 7.00	
	Increase	13 (6)	11 (16)	1.119	3.06	1.29 – 7.25	
Speed of change in amount of hard feed over last 28 days	No change	181 (89)	50 (74)	Ref.			0.01
	Sudden change	10 (5)	8 (12)	1.06	2.90	1.09 – 7.72	
	Gradual change	13 (6)	10 (15)	1.02	2.79	1.15 – 6.73	
Change in frequency of feeding hard feed in last 28 days	No change	190 (95)	56 (85)	Ref.			0.02
	Change in frequency	11 (6)	10 (15)	1.126	3.08	1.25 – 7.64	
Increased or decreased frequency of feeding hard feed in last 28 days	No change	194 (95)	62 (90)	Ref.			0.2
	Increased frequency	5 (3)	5 (7)	1.141	3.13	0.88 – 11.17	
	Decreased frequency	5 (3)	2 (3)	0.224	1.25	0.24 – 6.61	
Change in brand of hard feed in last 28 days	No	182 (91)	53 (79)	Ref.			0.02
	Yes	19 (10)	14 (21)	0.928	2.53	1.19 – 5.38	
Change in brand of hard feed in last 14 days	No	188 (94)	57 (85)	Ref.			0.04
	Yes	13 (7)	10 (15)	0.931	2.54	1.06 – 6.09	
Change in brand of hard feed in last 7 days	No	197 (98)	62 (93)	Ref.			0.05
	Yes	4 (2)	5 (8)	1.379	3.97	1.03 – 15.25	
Fed a supplement	No	66 (33)	29 (44)	Ref.			0.1
	Yes	136 (67)	37 (56)	-0.48	0.62	0.35 – 1.09	
Fed fruit or vegetables	No	168 (84)	57 (86)	Ref.			0.6
	Yes	22 (16)	9 (14)	-0.218	0.80	0.36 – 1.78	

Fed garlic	No	175 (87)	56 (85)	Ref.			0.6
	Yes	26 (13)	10 (15)	0.184	1.20	0.55 – 2.65	
Fed orthopaedic supplement	No	155 (77)	54 (82)	Ref.			0.6
	Yes	46 (23)	12 (18)	-0.289	0.75	0.37 – 1.52	
Fed probiotic supplement	No	190 (95)	61 (92)	Ref.			0.5
	Yes	11 (6)	5 (8)	0.348	1.42	0.47 – 4.24	
Saltlick available	No	185 (92)	59 (89)	Ref.			0.5
	Yes	16 (8)	7 (11)	0.316	1.37	0.54 – 3.50	
Oil supplemented in feed	No	179 (89)	53 (80)	Ref.			0.07
	Yes	22 (11)	13 (20)	0.691	2.00	0.94 – 4.23	
Fed calming supplement	No	197 (97)	66 (96)	Ref.			0.7
	Yes	7 (3)	3 (4)	0.246	1.28	0.32 – 5.09	
Fed at the same time as other horses	No	50 (29)	16 (27)	Ref.			0.7
	Yes	123 (71)	44 (73)	0.111	1.12	0.58 – 2.16	
Fed at the same time every day	No	9 (5)	5 (8)	Ref.			0.4
	Yes	168 (95)	55 (92)	-0529	0.59	0.19 – 1.83	
Water source in stable	Manual container	107 (74)	\40 (69)	Ref.			0.4
	Automatic drink	37 (26)	18 (31)	0.263	1.30	0.67 – 2.54	
Water source in field	Manual container	68 (38)	18 (32)	Ref.			0.74
	Automatic tank	103 (58)	38 (67)	0.332	1.39	0.74 – 2.64	
	Natural still water	3 (2)	0 (0)	-19.874	0.000		
	Natural running water	5 (3)	1 (2)	-0.280	0.756	0.08 – 6.88	

Manual container in field	No	115 (62)	39 (66)	Ref.			0.6
	Yes	71 (38)	20 (34)	-0.186	0.83	0.45 – 1.54	
Automatic tank in field	No	77 (41)	20 (34)	Ref.			0.3
	Yes	109 (59)	39 (66)	0.320	1.38	0.75 – 2.54	
Natural still water	No	180 (97)	58 (98)	Ref.			0.5
	Yes	6 (3)	1 (2)	-0.66	0.52	0.06 – 4.39	
Natural running water	No	179 (96)	58 (98)	Ref.			0.4
	Yes	7 (4)	1 (2)	-0.819	0.44	0.05 – 3.66	
Without water for more than two hours within previous 7 days	No	173 (85)	58 (88)	Ref.			0.6
	Yes	30 (15)	8 (12)	-0.229	0.80	0.35 – 1.83	

CHAPTER 4

Concluding discussion

The studies presented in this thesis represent the first major epidemiological investigations into large colon volvulus (LCV). This work has identified horse and management-level risk factors associated with increased risk of LCV, has described the pattern of long-term survival and has identified key prognostic indicators associated with survival following exploratory laparotomy and surgical correction of LCV. This novel research provides owners and clinicians with evidence-based information on the survival of horses following LCV, on factors that may assist in the recognition of horses at risk of LCV, and on factors that might be altered with the aim of reducing the incidence of LCV.

The study in Chapter 2 demonstrated that LCV of ≥360 degrees was associated with considerable post-operative mortality, with over 20% of horses not recovering from anaesthesia. Of the horses that recovered from anaesthesia, less than 50% were alive one year post-operatively, and only a third of horses were alive two years post-operatively. Median survival time in horses with a strangulating LCV that survived anaesthesia was only 365 days. This is in agreement with previous studies that have identified volvulus of ≥360 degrees to be strongly associated with poor survival and increased incidence of post-operative complications [6, 7].

Historically in the veterinary literature, there has been an over-emphasis on immediate post-operative success and a failure to account for mortality or complications that occur following discharge [153]. Cox proportional hazard models have been used in longitudinal colic studies as a means of identifying risk factors associated with long term survival in equine surgical colic cases, and, more specifically, in horses with small intestinal lesions, including horses with epiploic foramen entrapment, and in horses with large intestinal lesions [4, 6, 99, 100]. Future studies investigating the success of other surgical procedures should utilise survival analysis to evaluate long-term survival and post-operative complications, in order to produce accurate data on post-operative prognosis [153]. Relatively few studies have investigated the association of post-operative variables with long term survival following exploratory laparotomy, as was performed in Chapter 2 [100]. The identification of post-operative variables significantly associated with survival provides additional evidence that can be used when making clinical and financial decisions in the post-operative period.

Work presented in this thesis indicates that increased pre-operative packed cell volume (PCV), alteration in serosal colour intra-operatively, increased heart rate at 48 hours post-operatively, and colic during post-operative hospitalisation were significantly associated with decreased post-operative survival in horses with strangulating LCV. The association between pre-operative PCV and survival illustrates the importance of early referral, prior to alteration in PCV. This association has been previously identified in a number of studies [4, 6, 99, 100], highlighting the importance of this parameter as a prognostic indicator, and reinforcing the relationship of PCV with the degree of systemic inflammatory response. The identification of serosal colour as a key determinant of survival also emphasises the value of early referral and laparotomy, prior to severe vascular compromise of the colon. It has long been recognised that early referral and laparotomy improve prognosis in the equine colic patient. In addition, clinical parameters associated with a need for laparotomy are well established [154, 155, 156]. Despite this, delayed referral occasionally remains a factor implicated in mortality of the equine surgical colic patient. There is relatively limited published information on colic in first opinion equine practice [15, 157]. Further research is required on the incidence and management of colic in first opinion practice and on decision-making by the equine owner and equine clinician. Methods of further incorporating evidence based medicine during decision making in clinical practice merit future research in the veterinary field [158, 159, 160]. In human medicine the implementation of standard operating procedures in emergency cases has been shown to accelerate the time to achieve a diagnosis and to improve outcome [161, 162, 163]; the development of a standardised protocol for the management of colic in the horse might improve case management and treatment, particularly by the inexperienced clinician.

An improvement in our ability to treat pre- and post-operative systemic inflammatory response syndrome (SIRS) in horses with LCV would likely reduce post-operative morbidity and mortality. Recent interest has been shown in ethyl pyruvate, a derivative of pyruvate, which has anti-inflammatory properties [95, 96, 164]. Results have shown that ethyl pyruvate can be safely administered in horses [95, 96, 164]. In horses with experimentally lipopolysaccharide (LPS) induced endotoxaemia, ethyl pyruvate diminished pain and reduced the pro-inflammatory cytokine response [164]. The potential synergistic actions of ethyl pyruvate and flunixin meglumine, (a widely used and effective visceral analgesic and anti-inflammatory drug [39, 87, 88, 90]), in SIRS are worth exploring [164]. In addition efforts should continue to identify and develop other potential treatments for SIRS in the horse.

The study in Chapter 2 only included two horses that underwent a large colon resection and anastomosis (LCRA). LCRA has previously been associated with poor survival in horses with large intestinal disease undergoing surgery at the hospital studied in Chapter 2 [6]. However, Ellis *et al.* [11] demonstrated favourable survival in horses with LCV that underwent LCRA [11]. The population in the study by Ellis *et al.* varied considerably from the population described in Chapter 2, comprising of horses with a volvulus of ≥270 degrees, with a mean duration of colic signs prior to admission of only 4 hours [11]. Further international, collaborative research is necessary to ascertain whether LCRA improves the prognosis of horses with strangulating LCV. Ideally this would take the form of a randomised controlled trial (RCT), although this would present a variety of practical and ethical issues [165], which have largely precluded the use of RCTs when investigating veterinary surgical interventions.

It has been previously identified that the risk of post-operative colic is significantly increased in horses with LCV [7]. In the study presented in Chapter 2, nearly 20% of the population died as a result of colic following discharge from the hospital. Of the cases of LCV recruited in Chapter 3, over 50% of cases discharged from the hospital had a colic episode reported following discharge and a third of these horses were euthanased as a result. There is a paucity of research investigating the incidence and recurrence of post-operative colic following surgery for specific types of gastrointestinal lesions [15]. Survival analysis utilising a recurrent events model would allow further investigation of risk factors for postoperative colic following gastrointestinal surgery [137]. This work could be conducted utilising the data previously collected as part of the on going Colic Survival Study at the University of Liverpool. Description of the incidence and recurrence of post-operative colic, and risk factors for post-operative colic following specific surgical lesions would provide clinicians and owners further evidence based information when making clinical and financial decisions on the surgical colic patient.

None of the horses in this thesis underwent pexy of the large colon to the body wall, a procedure utilised in order to prevent recurrence of large colon displacement or volvulus. Colopexy is usually reserved for broodmares, or for horses used for low-level exercise [85, 86], due to the risk of colon rupture post-operatively in horses participating in athletic activity. This procedure can either be performed immediately following correction of the

LCV, or can be delayed so that the colon wall is no longer oedematous, potentially with the use of laparoscopy [166]. Further research is required to ascertain whether increased use of a colopexy procedure would reduce the incidence of post-operative colic and increase long-term survival following LCV.

Chapter 3 details the results of the first case-control study to investigate horse- and management-level risk factors for strangulating LCV. The study identified variables that may help with the recognition of horses at increased risk of LCV, has identified alterable management factors that might be modified in an attempt to reduce the incidence of LCV and has highlighted areas for further research.

Mares, especially those that had previously foaled, were found to be at increased risk of LCV. There is currently limited research detailing the incidence of colic in general, or the incidence of specific gastrointestinal lesions in the post-parturient mare [167]. Colic in the post-parturient period will not only effect the mare, but may also have an impact on the neonatal foal, and on conception rates, with financial and welfare implications. Further research investigating colic in general, and in particular LCV, in the post-parturient mare is required. A retrospective, descriptive study has been proposed utilising data from a leading Thoroughbred breeding group to describe the prevalence of colic in a multi-centre international population of Thoroughbred broodmares, and to investigate the prevalence of various gastrointestinal lesions within this population.

Horses that had exhibited quidding behaviour in the previous 90 days were at significantly increased risk of LCV. Quidding is often indicative of dental pathology, which has been previously found to increase the risk of SCOD and recurrent colic in the horse, and of large colon impaction in donkeys [15, 20, 117]. Further research is required to investigate the association of specific dental pathologies with various types of colic, and to investigate the cause of these associations. Reduced mastication of feed may lead to ingestion of longer length dietary fibre passing to distal portions of the gastrointestinal tract, increasing the risk of impaction [15, 150]. Alternatively dental pathology may lead to an alteration in the intestinal microbiota or the nutritional composition of ingesta reaching the large colon [15]. Effective education of horse owners, veterinarians and dental technicians regarding the provision of quality, regular dental care is important.

The study in Chapter 3 identified a history of multiple colic episodes within the preceding year to be associated with increased risk of LCV. These findings may suggest a sub-population of horses exists with underlying gastrointestinal dysfunction affecting the large colon. The density of the interstitial cells of Cajal (ICC) has been shown to be reduced in horses with large colon disorders compared to control horses. Further studies are required to elucidate mechanisms regulating ICC density and to assess the pathophysiological significance of these findings [43, 143]. Alternatively, it may be that inherent differences between individuals' intestinal microbiota predispose certain horses to recurrent colic and LCV [51]. Novel, culture-independent DNA sequencing technology has revolutionised the approach to the characterisation of human intestinal microbiome [168] and has opened new doors to the understanding of disease pathophysiology and the development of new treatment approaches [169]. In horses marked differences have been identified between the microbiome of healthy horses and those with colitis [169]. DNA sequencing technology may prove to be a valuable technique for the investigation of LCV. In addition, metabonomic technology could be used alone or in conjunction with metagenomic DNA sequencing to further investigate LCV in the horse. Metabonomic technology (including nuclear magnetic resonance and mass spectrometry) provides a sensitive and powerful tool to investigate the influence of genetic and environmental factors on a host's metabolic fingerprint [170, 171, 172]. This analytical method allows indirect measurement of intestinal bacterial activity through the quantification of metabolites produced by the host and by the intestinal microbiota [173, 174]. It has previously been used to investigate the effects of various intestinal diseases on the human metabolic phenotype and how the phenotype is altered by gastrointestinal surgery [175, 176, 177, 178]. Work is currently being undertaken to characterise the metabolite profiles of equine biofluids, including urine, plasma and faeces, at Imperial College London and the University of Liverpool. The use of metabonomic technology in conjunction with multivariate statistical analysis could provide a highly suitable method to further investigate risk factors for LCV in the horse, allowing exploration of metabolic differences between control horses and those that have suffered LCV or recurrent colic, and allowing research into the effect of diet, foaling, or dental pathology on the equine metabolic profile.

In Chapter 3 a number of risk factors relating to feeding types and practices were identified. However, difficulty was encountered in obtaining accurate information on the weight of concentrate and forage fed, and on the nutritional composition of concentrate, forage, and pasture fed. Further investigation, for example utilising metabonomic technology, is required to investigate the association of more specific nutritional variables with equine colic in general, and with LCV.

The evidence from the study performed in Chapter 3 suggests there may be a number of management practices that could be implemented to reduce the risk of LCV in high-risk individuals:

- Avoiding sudden increases in the duration of stabling and maximising turnout
- Minimising the number of individuals involved with an individual horse's care
- Minimising the number of horses on a premises or managing horses in smaller groups
- Providing quality, regular dental care
- Avoid feeding sugar-beet
- Feeding haylage or grass rather than hay
- Avoiding sudden changes in the amount of forage fed

An intervention study, potentially in high-risk individuals, such as a population of broodmares, would be required to determine if modification of these factors reduces the incidence of LCV.

As with all epidemiological research the validity of the studies presented in this thesis relates to the absence of systematic bias in the study design and results [179]. In Chapter 2, horses were selected for inclusion in the study if they were referred to the participating hospital and underwent general anaesthesia and exploratory laparotomy. The necessity to exclude those horses euthanased without an opportunity for exploratory laparotomy, either prior to, or following referral, due to an extremely poor prognosis, will have influenced the survival rates and analysis. One could hypothesise that inclusion of theses horses would further increase post-operative mortality. In addition, inclusion of horses euthanased prior to travel might influence the variables associated with survival; in some cases the degree of pain may preclude safe transportation to a referral centre and the presence or absence of severe pain might be significantly associated with survival if these horses were included. Horses that were euthanased on the operating table were included in the study population; it was deemed their inclusion would provide clinicians and owners with more accurate information on the prognosis of horses presenting with a strangulating LCV. However, the inclusion of horses euthanased on the operating table potentially allows a surgeon's judgment and the owner's financial situation to have a potential influence on survival time.

Misclassification bias was minimised in both studies by ensuring recruitment of cases only from clinicians experienced in performing exploratory laparotomy. Furthermore in Chapter 2, where the case definition was retrospective, only horses with a volvulus of greater than or equal to 360 degrees were included to ensure all cases of volvulus were strangulating in nature. In Chapter 3 the inclusion criteria was that the volvulus was strangulating in nature, and of greater than or equal to 270 degrees; this was deemed suitable as the study in Chapter 3 was prospective, and it was felt that this criteria would include all cases of strangulating volvulus. Adequate and complete follow-up is a prerequisite for the conduct of any survival study [25]. The 30-month period during the study in Chapter 2 where follow-up questionnaires were suspended, due to an alteration in funding, may have reduced the accuracy of survival time (recall bias) and increased the number of censored individuals.

In Chapter 3 selection bias was minimised by using a population of non-colic controls that were representative of the population at risk [179]. In an attempt to ensure controls would become cases should the outcome (LCV) occur, control clients were asked whether the horse would, if deemed necessary, undergo colic surgery. Random selection of a horse that was owned or cared for by a control client also reduced selection bias, by removing the potential for clients to select a horse they felt was of particular interest. Whilst the number of clients selected as controls from each clinic was proportional to the caseload of that clinic, the long-standing reputation of Hospital 3 for referrals for colic surgery may have resulted in referral bias; horses that would have been referred to another hospital had they suffered from a different disease process, may have been referred to Hospital 3 with gastrointestinal disease. This would explain the increased risk of LCV associated with Hospital 3 identified in Chapter 3. Alternatively the increased risk associated with Hospital 3 might have been because more cases of LCV were reported at this hospital, since the study's author was based at Hospital 3.

Diminishing response rates increase the risk of sampling bias [180]. The study in Chapter 3 achieved high response rates (completion of the questionnaire in 73% of controls and 97% of cases). This may have been due to postal contact describing the study preceding telephone calls, calls at different times of the day if there was no answer on initial contact, and informing owners that the study was investigating colic in the horse. Horse owners perceive colic to be an important disease in the horse [181] and it was hoped that providing this information would incentivise cases and controls to participate in the study. However, informing owners the study was investigating colic may have influenced their responses and therefore resulted in bias. The greater response rate in cases, compared to controls, is likely to be due to the increased motivation of owners whose horses had undergone colic surgery, to participate in research on this topic. This may have introduced a degree of sampling bias. Telephone questionnaires were used to collect data from cases and controls in order to reduce this effect; direct contact has been shown to improve response rates compared to self-administered questionnaires [152]. In human medicine, postal surveys, when compared to telephone surveys, produce less reliable responses to questions relating to environmental exposures, and increase item omission in response to questions [182]. Recall bias was minimised in Chapter 3 by ensuring questionnaires were conducted as soon as possible after the date of surgery in cases, to avoid memory decay [183]. In addition the majority of questions related only to the horse's health and management in the previous 4 weeks.

Extrapolation of the findings in Chapter 2 to other populations must be done with caution since the study was conducted at only one UK equine hospital. The majority of this hospital's caseload is made up of pleasure horses, and care should be taken generalising the findings to other populations, particularly in areas where valuable broodmares, race or performance horses predominate. In Chapter 2 the median duration of colic signs prior to admission was 10 hours. It is likely the duration of colic signs prior to referral would be reduced in a dense thoroughbred breeding area, which would influence the degree of pre-operative cardiovascular compromise and therefore post-operative survival. In addition survival is likely to be influenced by surgical expertise; the long-standing reputation for colic surgery and specialist status of the majority of the hospital's surgeons in Chapter 2 may make the results less applicable to other centres with lesser expertise. Follow-up on the cases included in Chapter 3 is on going, in order to allow survival and post-operative complications to be assessed in a larger, multi-centre population.

In Chapter 3 the multi-centre nature of the research increased the number of cases collected during the study period and allowed investigation of risk factors for LCV in the UK horse population, rather than in one referral population. However, the geographical location and caseload of the four hospitals differ, with two of the hospital populations' consisting predominantly of thoroughbreds and competition horses, and two of the hospital populations' consisting largely of pleasure horses. Risk factors associated with LCV in the UK may alter depending on the population of horses or geographical region investigated. In addition, the results of the study may not be applicable outside the UK; worldwide differences in genetics, management practices, nutrition and meteorological conditions may influence risk factors for LCV.

In conclusion, the studies within this thesis have improved our ability to prognosticate survival in horses with LCV and our ability to identify individuals at increased risk of this disease. Importantly, the work has also identified a number of management practices, which could be modified, in an attempt to reduce the incidence of LCV. This thesis has highlighted several areas that merit on-going research, which may, in the future, further contribute to our understanding of the disease. Results from this thesis will be relevant to both clinicians and horse owners, particularly owners whose horses undergo surgery to correct a LCV, and owners of horses at risk of LCV.

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GENERAL APPENDIX

General Appendix

Table of contents

Please note: Some of the documents within this appendix have been reduced in size from the original A4 format

Appendix 1: *Material published during the writing of this thesis.*

This text box is where the unabridged thesis included the following third party copyrighted material:

Suthers, J. M., Pinchbeck, G. L., Proudman, C. J. and Archer, D. C. (2013), Survival of horses following strangulating large colon volvulus. Equine Veterinary Journal, 45: 219–223. doi: 10.1111/j.2042-3306.2012.00620.x

This text box is where the unabridged thesis included the following third party copyrighted material:

Suthers, J. M., Pinchbeck, G. L., Proudman, C. J. and Archer, D. C. (2013), Risk factors for large colon volvulus in the UK. Equine Veterinary Journal. doi: 10.1111/evj.12039

Appendix 2: *Information leaflet sent to randomly selected control client.*

If you have any queries or questions about the project please contact:

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UNIVERSITY OF LIVERPOOL

Information sheet v1.2 Jan 10

UNIVERSITY OF LIVERPOOL

COLIC STUDY

Epidemiology of Large Colon Torsion

Based at Liverpool University in collaboration with:
Bell Equine Veterinary Clinic
Donnington Grove Veterinary Surgery
Rossdales Equine Hospital

WHAT IS COLIC?

Colic is a word used to describe any form of abdominal pain. In the majority of cases this pain is related to the gastro-intestinal tract. There are several parts of the gastro-intestinal tract that can be affected to varying degrees of severity. These include the stomach, the small intestine, the small colon and the large colon, which is the area of interest in this study.

IS SURGERY ALWAYS REQUIRED?

In the majority of cases of colic surgery is not required. The colic will either resolve spontaneously or respond to medication administered by your vet. Unfortunately a few cases of colic do not respond to this treatment and may be fatal unless surgery is performed. Colic surgery is a major operation and cases can require intensive post-operative care and a long recovery period.

WHAT IS EPIDEMIOLOGY AND WHAT DOES THIS STUDY INVOLVE?

Epidemiology is the study of factors affecting the health and disease of populations. Studies like this one allow us to identify factors that may increase or decrease the risk of a disease occurring. Identification of risk-factors for colic will enable us to advise horse owners and vets on how to reduce the risk of certain types of colic occurring.

Information sheet v1.2 Jan 10

We are investigating a type of colic called **large colon torsion.**

IF YOUR HORSE HAS HAD SURGERY AND BEEN DIAGNOSED WITH A LARGE COLON TORSION...

We will contact you to invite you to participate in the study. If you are willing to participate we will ask you some questions about your horse or pony. This questionnaire will take about ten minutes to complete and will be conducted over the telephone. If we call you at an inconvenient time we can arrange to phone back at a time that suits you. **Your participation is entirely voluntary and you are free to leave the study at any time, without providing an explanation. All information collected will be strictly confidential and will be stored securely.**

IF YOUR HORSE IS BEING USED AS A CONTROL (COMPARISON) AND HAS NOT BEEN DIAGNOSED WITH A LARGE COLON TORSION.....

We will contact you in the next couple of weeks to invite you to participate and, if you are willing, ask you questions about a randomly selected horse or pony. This will be done over the telephone at a time convenient for you and will take about ten minutes.

Your participation is entirely voluntary and you are free to leave the study at any time, without providing an explanation. All information collected will be strictly confidential and will be stored securely.

Appendix 3.1: *Recording form for notifying primary investigator of cases by collaborating clinics.*

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EPIDEMIOLOGY OF LARGE COLON TORSION

CASE NOTIFICATION

HOSPITAL NAME...

CLINICIAN WHO CAN BE CONTACTED ABOUT THE CASE

....

CASE NAME...

HOSPITAL CASE NUMBER..

OWNER / CARER NAME...

ADDRESS...

....

....

OWNER / CARER CONTACT TELEPHONE NUMBER(S)

....

DATE OF SURGERY..

IS THE HORSE STILL ALIVE?...

DATE OF EUTHANASIA (If diagnosed at post mortem).......................................

PLEASE Fax: FAO: Jo Suthers: 0151 794 6034

E-mail: jsuthers@liverpool.ac.uk

Tel: 0151 794 6041

HORSERACE BETTING HBLB LEVY BOARD

Appendix 3.2: *Recording form for recording case details on presentation and exploratory*

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HORSERACE BETTING LEVY BOARD HBLB

SURVIVAL DATA - LARGE COLON TORSION PROJECT

We would be really grateful if you could complete the following as fully as possible for all cases of large colon torsion (torsion >180) identified at surgery or post-mortem.
Thank you so much for your help!

Please fax: FAO Jo Suthers
Fax No: 0151 794 6034

Or scan and e-mail Jo
E-mail: jsuthers@liverpool.ac.uk

HORSE / PONY NAME: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

OWNER SURNAME: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

SURGERY DATE: ☐☐ / ☐☐ / ☐☐

CLINIC:
- ☐ Bell Equine
- ☐ Donnington Grove
- ☐ PLEH
- ☐ Rossdales

Study No: ☐☐☐☐

CLINICAL PRESENTATION ON ADMISSION

Duration of colic on admission: ☐☐ Hours

Pre-operative Heart Rate : ☐☐☐ bpm

Pre-operative PCV (%): ☐☐ %

Pre-operative Total protein: ☐☐ g/L

Pre-operative Blood Lactate: (If acquired) ☐☐ . ☐☐ mmol/L

Peritoneal Fluid Total Protein: (If acquired) ☐☐ g/L

Peritoneal Fluid Lactate: (If acquired) ☐☐ . ☐☐ mmol/L

Please turn over

43980

SURGERY

Degree of torsion: ☐ >180 <270 ☐ >270 <360 ☐ >360 >720

Duration of Surgery: ☐☐☐ Mins *(Defined as time from first skin incision to skin closure)*

Was a resection performed?: ☐ No ☐ Yes

Degree of large colon oedema: ☐ None ☐ Mild ☐ Moderate ☐ Severe

Colour of Serosa: ☐ Normal ☐ Red ☐ Purple

Colour of Mucosa: ☐ Normal ☐ Red ☐ Black

Mucosal Bleeding at enterotomy site: ☐ Normal ☐ Reduced ☐ Absent

POST-OPERATIVE PROGRESS

Heart Rate 24 hours post surgery: ☐☐☐ bpm

Heart Rate 48 hours post surgery: ☐☐☐ bpm

Heart Rate 72 hours post surgery: ☐☐☐ bpm

Total protein 24 hours post surgery: ☐☐ g/L PCV 24 hours post surgery: ☐☐ %

Total protein 48 hours post surgery: ☐☐ g/L PCV 48 hours post surgery: ☐☐ %

Total protein 72 hours post surgery: ☐☐ g/L PCV 72 hours post surgery: ☐☐ %

Did the horse colic in the 72 hour post-operative period?: ☐ No ☐ Yes

Was a re-laparotomy performed? ☐ No ☐ Yes
(Defined as repeat surgery performed within 7 days of first surgery)

Date of discharge from hospital: ☐☐ / ☐☐ / ☐☐

OR Date of death / euthanasia: ☐☐ / ☐☐ / ☐☐

If died / euthanased reason for death / euthanasia:

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laparotomy.

Appendix 4: *Telephone questionnaire conducted with cases and controls.*

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HBLB HORSERACE BETTING LEVY BOARD

EPIDEMIOLOGY OF LARGE COLON TORSION QUESTIONNAIRE

HORSE / PONY NAME ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

HOSPITAL
- ☐ BELL EQUINE
- ☐ DONNINGTON GROVE
- ☐ PLEH
- ☐ ROSSDALES

CONTROL / CASE
- ☐ CONTROL
- ☐ LARGE COLON TORSION

STUDY NUMBER ☐☐☐☐

DATE OF INTERVIEW ☐☐ / ☐☐ / ☐☐☐☐

QUESTIONNAIRE TIME ☐☐ : ☐☐
Use 24 hour clock

DURATION OF INTERVIEW ☐☐ Mins

DAYS BETWEEN QUESTIONAIRE ADMINISTRATION AND TIME OF INTEREST ☐☐

OWNER NAME

☐☐☐☐ ☐ ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

STREET / ROAD

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

VILLAGE

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

TOWN / CITY

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

COUNTY

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

POSTCODE

☐☐☐☐ ☐☐☐

TELEPHONE NUMBERS

☐☐☐☐☐☐☐☐☐☐☐ ☐☐☐☐☐☐☐☐☐☐☐

E-MAIL ADDRESS

☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

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SECTION A: HORSE DETAILS

A1. Are you the owner / carer / trainer of (name of horse =X)? ☐ Owner ☐ Carer ☐ Trainer

A2. How long has (X) been in your care? ☐☐ Years ☐☐ Months

A3. What gender is (X)? ☐ Stallion ☐ Gelding ☐ Mare

A4. How old is (X)? ☐☐ Years ☐☐ Months

A5. What breed is (X)? ________ Code ☐☐

A6. What height is (X)? ☐☐ . ☐ hh ☐☐☐ . ☐ cms

A7. How would you describe his / her body condition?

☐ Very lean ☐ Lean ☐ Normal ☐ Overweight ☐ Very Overweight

A8. What is (X) mainly used for?

________ Code ☐☐

A9. Is (X) currently competed? ☐ Yes ☐ No

If yes, please specify which discipline(s)? ________ Code ☐☐

At what level is (X) competing? ________ Code ☐☐

A10. Is (X) insured for vets fees? ☐ Yes ☐ No

SECTION B: PREVIOUS MEDICAL HISTORY

B1: Has (X) ever had colic previously? ☐ Yes ☐ No **If No continue to question B2.**

If yes

How long since the last episode of colic? ☐☐ Years ☐☐ Months ☐☐ Days

How many episodes of colic have occurred within the last three years? ☐☐

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How long ago did these episodes occur?

Episode 1 - Most recent	☐ Years	☐☐ Months	☐☐ Days
Episode 2	☐ Years	☐☐ Months	☐☐ Days
Episode 3 - Least Recent	☐ Years	☐☐ Months	☐☐ Days

Further episodes

Episode 1 (Most recent)

☐ Did not require veterinary attention
☐ Required veterinary attention but no medical treatment
☐ Required medical treatment
☐ Required surgical intervention with or without previous medical treatment

Do you know what caused the colic episode?

Episode 2

☐ Did not require veterinary attention
☐ Required veterinary attention but no medical treatment
☐ Required medical treatment
☐ Required surgical intervention with or without previous medical treatment

Do you know what caused the colic episode?

Episode 3 (Least recent)

☐ Did not require veterinary attention
☐ Required veterinary attention but not medical treatment
☐ Required medical treatment
☐ Required surgical intervention with or without previous medical treatment

Do you know what caused the colic episode?

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B2. Has (X) ever had abdominal surgery? ☐ Yes ☐ No ☐ Don't Know

If yes

How long ago was the surgery undertaken? [ ][ ] Years [ ][ ] Months [ ][ ] Days

What was found at surgery (If known)?

B3. Has (X) had any medical problems over the last 4 weeks? ☐ Yes ☐ No ☐ Don't Know

If yes details?

Code [ ][ ]

B4. Has (X)received any medication (other than routine worm control) over the last 4 weeks?

☐ Yes ☐ No ☐ Don't Know

If yes

What medication?

Code [ ][ ]

How long did (X) receive this medication for? [ ][ ] Days

How long ago did this medication stop being administered? [ ][ ] Days

B5. Has (X) been seen by a vet for any reason other than colic in the last 7 days?

☐ Yes ☐ No ☐ Don't Know

If yes

Reason for visit?

Code [ ][ ]

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SECTION C: BREEDING HISTORY (If mare)

C1. Has she ever been used as a brood mare?

☐ Never ☐ One foal ☐ More than one foal ☐ Don't know

If used as a brood mare

C2. How long since she last foaled? [ ][ ] Years [ ][ ] Months [ ][ ] Days

C3. Has she ever had colic in the six month period after foaling?

☐ Yes ☐ No ☐ Don't know

If yes How long after foaling? [ ] Months [ ][ ] Days

C4. Has she ever had colic in the three month period before foaling?

☐ Yes ☐ No ☐ Don't know

If yes How long before foaling? [ ] Months [ ][ ] Days

C5. Is she in foal at present? ☐ Yes ☐ No ☐ Don't Know

If yes
When is she due to foal? [ ] Days to foaling [ ][ ][ ]

SECTION D: PREMISES

D1. What type of premises is (X) kept on?

☐ Field or Pasture
☐ Private yard
☐ Livery yard
☐ Profesional /competition yard
☐ Riding school
☐ Stud Farm
☐ Other [ ]

D2. How long has (X) been on the current premises? [ ][ ] Years [ ][ ] Months [ ][ ] Days

If less than 12 months

How many premises has (X) been on over the last 12 months? [ ]

D3. How many horses are on the current premises? [ ][ ][ ]

D4. How many people care for X? [ ]

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D5. Who is the main carer?

- ☐ Owner
- ☐ Loaner
- ☐ Owner / loaner's relative or spouse
- ☐ Owner / loaner's friend
- ☐ Profesional groom
- ☐ Other:

Who are the other carers?

- ☐ Owner
- ☐ Loaner
- ☐ Owner / loaner's relative or spouse
- ☐ Owner / loaner 's friend
- ☐ Professinal groom
- ☐ Other:

SECTION E: STABLING

E1. How many hours a day is (X) currently stabled? Hours

E2. How many hours a day is (X) currently turned out? Hours

E3. Has there been a change in the number of hours (X) has been stabled in the last 4 weeks? ☐ Yes ☐ No

If yes How long ago did this change occur? days ago

What type of change was this? Code

E4. What type of bedding is (X) currently on?

- ☐ N/A (Not stabled)
- ☐ Straw
- ☐ Hemp
- ☐ Shavings
- ☐ Paper / Cardboard
- ☐ Rubber matting only
- ☐ Other:

E5. Has the type of bedding changed at all over the last 4 weeks? ☐ Yes ☐ No

If yes How long ago did this change occur? Days ago

What type of change was this? Code

E6. Does (X) ever eat their bedding?

☐ No ☐ Yes, on previous bedding ☐ Yes, on current bedding ☐ N/A

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SECTION F: PASTURE / TURNOUT

F1. What type of area is (X) turned out into?
☐ Not turned out
☐ Yard
☐ Arena
☐ Grass field
☐ Other:

F2. How would you describe the condition of the current pasture in which (X) has been turned out?
☐ N/A, not turned out ☐ Short grass ☐ Mid-length grass ☐ Long grass

F3. Is the soil type where (X) is turned out sandy? ☐ Yes ☐ No ☐ N/A ☐ Don't Know

F4. Has (X) been moved to a different pasture within the last 4 weeks?

☐ Yes ☐ No ☐ N/A ☐ Don't know

If yes how many different pastures?

If yes has the quality of the pasture changed? ☐ Yes ☐ No

If yes has the quality of the pasture ☐ Improved? ☐ Deteriorated?

SECTION G: NUTRITION

G1. Currently, how many times a day is (X) fed forage?
☐ None
☐ Fed every other day or less
☐ Once daily
☐ Twice daily
☐ Three times daily
☐ Fed ad-lib
☐ Other:

G1a Are there times of the day when (X) has finished all of their forage?
☐ Yes ☐ No ☐ Don't Know

G2. What type of forage is (X) curently fed?
☐ None - grass only
☐ Soaked hay
☐ Dry hay
☐ Haylage
☐ Horsehage
☐ Other:

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G3. Has the amount of forage (X) is fed changed in the last 4 weeks?

☐ Yes ☐ No ☐ N/A, not fed forage ☐ Don't know

If yes
How has it changed? ☐ Increase ☐ Sudden (over a few days)
☐ Decrease ☐ Gradual (over several weeks)

When did this change start? [ ][ ] Days ago

G4. Has the frequency of forage (X) is fed changed in the last 4 weeks?

☐ Yes ☐ No ☐ N/A ☐ Don't know

If yes How has it changed?
☐ Increased frequency
☐ Decreased frequency
☐ Varies on a regular basis
☐ Altered frequency for a few days only

When did this change start? [ ][ ] Days ago

G5. Has the type of forage fed changed at all over the last 4 weeks?

☐ Yes ☐ No ☐ N/A ☐ Don't know

If yes Type of change? [ ] Code [ ][ ]

When did this change start? [ ][ ] Days ago

G6. Has the batch of forage (X) is fed changed in the last 4 weeks?

☐ Yes ☐ No ☐ N/A ☐ Don't know

If yes When did this change occur? [ ][ ] Days ago

G7. How many times a day is (X) fed bucket feed (mix / nuts / straights / chaff)?

☐ None
☐ Fed every other day or less
☐ Once daily
☐ Twice daily
☐ Three times daily
☐ Four times daily
☐ More than four times daily
☐ Other: [ ]

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G8. What types of concentrates are fed? (If not fed concentrates go to question G12)

Type and Brand Name	Times of day fed	Aprox amount fed

Mix / Nuts

☐ Yes ☐ No ☐ Don't know No of times fed / day ☐ Aprox Amount ☐☐.☐☐ KG

Brand Name and type:

Code ☐☐

Whole Grain

☐ Yes ☐ No ☐ Don't know No of times fed / day ☐ Aprox Amount ☐☐.☐☐ KG

Brand Name and type:

Code ☐☐

Sugarbeet pulp

☐ Yes ☐ No ☐ Don't know No of times fed / day ☐ Aprox Amount ☐☐.☐☐ KG

Brand Name and type:

Code ☐☐

Roughage (Short fibre)

☐ Yes ☐ No ☐ Don't know No of times fed / day ☐ Aprox Amount ☐☐.☐☐ KG

Brand Name and type:

Code ☐☐

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G9. Has the amount of concentrates (X) is fed changed in the last 4 weeks?

☐ Yes ☐ No ☐ N/A ☐ Don't know

If yes

How has it changed?
- ☐ Gradual increase (over several weeks)
- ☐ Sudden increase (over a few days)
- ☐ Gradual decrease (over several weeks)
- ☐ Sudden decrease (over a few days)
- ☐ Varies on a regular basis

When did this change occur? ☐☐ Days ago

G10. Has the frequency of feeding of concentrates (X) is fed changed in the last 4 weeks?

☐ Yes ☐ No ☐ N/A ☐ Don't Know

If yes

How has it changed?
- ☐ Increased frequency
- ☐ Decreased frequency
- ☐ Varies on a regular basis

When did this change occur? ☐☐ Days ago

G11. Has the type of concentrates fed (including brand / manufacturer) changed at al in the last 4 weeks?

☐ Yes ☐ No ☐ N/A ☐ Don't know

If yes

Type of change? ________ Code ☐☐

When did this change occur? ☐☐ Days ago

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G12. Is (X) fed any supplements?

☐ Yes ☐ No ☐ Don't know

If yes, what supplements are fed?

Vegetables / Fruit	☐ Yes	☐ No	☐ Don't know
Garlic / Other herbal	☐ Yes	☐ No	☐ Don't know
Ortho supplement	☐ Yes	☐ No	☐ Don't know
Pre / Probiotics	☐ Yes	☐ No	☐ Don't know
Salt / Mineral Lick	☐ Yes	☐ No	☐ Don't know
Oil	☐ Yes	☐ No	☐ Don't know
Other			

G13. Are all the horses on the yard fed at the same time?

☐ Yes ☐ No ☐ Don't know ☐ N/A

G14. Is (X) fed at the same time every day (within an hour)?

☐ Yes ☐ No ☐ Don't know ☐ N/A

G15. What is (X's) water source?

STABLE	FIELD
☐ Manually filled container	☐ Manually filled container
☐ Automatic drinker	☐ Automatic tank
☐ Both	☐ Natural running water
☐ Other	☐ Natural still water
☐ Not stabled	☐ Not turned out

G16. Due to, for example, transport, weather conditions or contamination could (X) have been without water for more than two hours within the last 7 days?

☐ Yes ☐ No ☐ Don't know

If yes why?

Code

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SECTION H: EXERCISE AND TRANSPORT

H1. Is (X) currently in routine work? ☐ Yes ☐ No

If yes

Currently how many times a week is (X) exercised? ☐☐

Currently how many hours exercise is (X) doing per week?

☐☐.☐ hours / week

What sort of evercise is performed most of the time?

☐ Light exercise (i.e. Light hacking or schooling)

☐ Modorate exercise (i.e. Schooling, hacking and light competition work)

☐ Hard exercise (i.e. Some fast work, regular competition / training)

☐ Very hard exercise (i.e. In training for racing / eventing / endurance)

H2. Has the duration of exercise changed in the last 4 weeks?

☐ Yes - Gradually (over several weeks)
☐ Yes - Suddenly (over a few days)
☐ No
☐ Don't know

If yes, has the duration ☐ Increased? ☐ Decreased?

Reason for change ______ Code ☐☐

When did this change occur? ☐☐ Days ago

H3. Has the intensity of exercise changed in the last 4 weeks?

☐ Yes - Gradually (over several weeks)
☐ Yes - Suddenly (over a few days)
☐ No
☐ Don't know

If yes, has the intensity ☐ Increased? ☐ Decreased?

Reason for change ______ Code ☐☐

When did this change occur? ☐☐ Days ago

H4. Has (X) been ridden within 2 hours of feeding in the last 48 hours?

☐ Yes ☐ No

If yes Was this ☐ Before feeding? ☐ After feeding?

Is (X) routinely ridden within 2 hours of feeding ? ☐ Yes ☐ No ☐ Don't Know

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H5. Has (X) been transported in the last 7 days? ☐ Yes ☐ No

If yes How many times has (X) been transported in the last 7 days (there and back = 2)? ☐

Total duration of transport? ☐☐ Hours

No of days since last transported? ☐☐ Days

How long was this last journey? ☐☐ Hours

SECTION I: HORSE BEHAVIOUR

I1. Is (X) restless when stabled? ☐ Yes, is restless ☐ No, is settled ☐ Don't know ☐ N/A

I2. If left in without other horses does (X) become distressed?

☐ Yes, becomes distressed ☐ No, not worried ☐ Don't know ☐ N/A

I3. How does (X) react just before being fed?

☐ Becomes very agitated
☐ Shows interest, looks over door but does not become overly excited
☐ Shows little or no interest
☐ Don't know
☐ N/A

I4. How quickly does (X) eat concentrates?

☐ Very quickly - bolts feed
☐ Eats all feed at nomal speed
☐ Eats slowly, picks at feed
☐ Don't know
☐ N/A

I5. How quickly does (X) eat forage?

☐ Very quickly - eats all forage immediately
☐ Eats all forage but not at once
☐ Doesn't eat all forage
☐ Don't know
☐ N/A

I6. If (X) is stressed (e.g. moves to a newpremises or following transport / competition) do you see any change in feeding patterns?

☐ Yes, will go off feeds ☐ No, eats as normal ☐ Don't know ☐ N/A

I7. Can (X) see other horses from his / her stable? ☐ Yes ☐ No ☐ Don't know ☐ N/A

I8. Is (X) able to have direct physical contact with another horse or pony on a regular basis (e.g. turned out together or able to groom each other over a fence)? ☐ Yes ☐ No ☐ Don't know ☐ N/A

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I9. Does (X) ehibit any stereotypic behaviour or vices? (If "No" go onto section J)

Crib-bite or windsuck?	☐ Yes	☐ No	☐ Don't know
Box walk?	☐ Yes	☐ No	☐ Don't know
Weave?	☐ Yes	☐ No	☐ Don't know
Wood chew?	☐ Yes	☐ No	☐ Don't know
Other:			

I10. When is this behaviour / are these behaviours seen?

☐ Only when stabled
☐ Only when turned out
☐ Both when stabled and turned out
☐ Other:

I11. How often is this behaviour seen?

☐ Seen every day for prolonged periods
☐ Seen every day but for short periods
☐ Seen at least once a week but not every day
☐ Seen on rare / specific occasions
☐ Not currently seen e.g. wearing a collar / kept out

I12. In relation to being fed when do you see this behaviour?

☐ Not seen before, during or after feeding
☐ Seen before feeding but not during or after
☐ Not seen before but observed during or after
☐ Seen before, during and after feeding
☐ Don't know

I13. Does this behaviour / do these behaviours change at feeding time?

☐ Yes, exhibited more often / for longer periods
☐ Yes, decreased - exhibited less often / for shorter time periods
☐ No change - behaviour remains at the same intensity / frequency
☐ Don't know

I14. Do you take any measures to try to stop any of these behaviours from occurring?

☐ Yes ☐ No ☐ Don't know ☐ N/A

If yes What measures are taken? Code

Do they work? ☐ Yes, completely ☐ Yes, to some extent ☐ No ☐ Don't know

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SECTION J: ROUTINE HEALTH CARE

J1. Do you know when (X) was last wormed? ☐ Yes ☐ No

If yes, how long ago was this? ☐ Years ago ☐☐ Months ago ☐☐ Days ago

J2. Do you know what product was used? ☐ Yes ☐ No

If yes, what product was used? ☐☐ Code

J3. Has (X) been wromed with a double dose of pyrantel (Strongid P / Pyratape / Equitape) or praziquantel (Equimax, Equest Pramox, Eqvalan Duo) in the last six months?

☐ Yes ☐ No ☐ Don't know

J4. Has (X) been wormed with ivermectin (Equimax, Bimectin, Exodus, Eqvalan, Eraquell, Vectin) or moxidectin (Equest, Equest Pramox) in the last twelve months?

☐ Yes ☐ No ☐ Don't know

J5. When did (X) last have their teeth rasped / checked?

☐☐ Years ago ☐☐ Months ago ☐☐ Days ago

J6. Who was this done by?

☐ Veterinary surgeon
☐ Equine Dentist
☐ Both of above
☐ Other
☐ Don't know

J7. Was a dental gag used? ☐ Yes ☐ No ☐ Don't know

J8. Was a light source used? ☐ Yes ☐ No ☐ Don't know

J9. Were any abnormalities noted? ☐ Yes ☐ No ☐ Don't know

If yes, what abnormalities were noted? ☐☐ Code

J10. Have you seen (X) dropping their feed when they eat in the last 3 months?

☐ Yes ☐ No ☐ Don't know

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Can you detail any changes that have occurred in your horse's management (including feeding, exercise, stabling and turnout) over the last 48 hours......

Thank you very much for you participation with this project.

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